Synthesis of New Cu Complex Based on Natural 5Z,9Z-Eicosadienoic Acid: Effective Topoisomerase I Inhibitor and Cytotoxin against the Cisplatin-Resistant Cell Line

Lilya U. Dzhemileva,* Vladimir A. D’yakonov, *Leisan K. Dil’mukhametova, and Usein M. Dzhemilev

Institute of Petrochemistry and Catalysis of RAS (IPC RAS), Prospect Octyabrya, 141, 450075 Ufa, Russian Federation

ABSTRACT: The complex \((\text{bipy})_2\text{Cu}(5,9\text{-eicd})\) was prepared by the reaction of \(\text{Cu(OAc)}_2\) with \(5Z,9Z\)-eicosadienoic acid and 2,2’-bipyridine in methanol. The new copper complex showed high antitumor activity in vitro toward A2780cis, A2780, Hek293, K562, HL60, Jurkat, and U937 cell lines and efficiently inhibited human topoisomerase I. Using flow cyttofluorometry, \((\text{bipy})_2\text{Cu}(5,9\text{-eicd})\) was studied for the effect on the cell cycle and apoptosis-inducing activity in tumor cells.

1. INTRODUCTION

In 1969, Rosenberg and co-workers reported the first results concerning the cytotoxicity of platinum compounds against murine tumors, which initiated a new area of medicinal chemistry dealing with metal-containing antitumor agents. Currently, platinum-based compounds such as cisplatin, carboplatin, and oxaliplatin are widely used in medical practice. Although cisplatin and its derivatives have been successfully included into the armory of anticancer chemo-therapeutical agents, there are problems related to their high toxicity and low selectivity to malignant tumors and, in some cases, drug resistance of cancer cells. This stimulates extensive studies aimed at the development of new metal derivatives that would be free from the indicated drawbacks. Among non-platinum compounds, researchers are focused on less toxic copper complexes. The properties exhibited by complexes are substantially dependent on the nature of ligands and the donor atoms that are coordinated to the metal.

The relationship between the structure and biological activity of copper complexes has been investigated for as long as more than 5 decades. The biological activities of many copper compounds are governed by the chelating properties of the ligands towards transition-metal ions. It is also known that metal-ion chelates show a much better uptake by living organisms than free metal ions. However, many issues concerning the behavior of copper ions in the intracellular space remain unsolved: it is absolutely unknown whether or not the structure of sophisticated complexes is retained in the cell as the copper ion is released and what happens when the copper valence changes from Cu(II) to Cu(I). An increase in the copper-ion concentration inside the cells is known to induce general intoxication to inhibit the DNA synthesis and oxidative phosphorylation, and also to result in total thiol oxidation to disulphides. The biological effects of copper complexes are highly diversified and selective, and all this complicates the search for and systematization of literature sources. All of the listed issues can apparently attest to the high diversity of biological effects of these compounds.

A fairly important factor for the design of modern antitumor compounds is the selection of the molecular target, most often, an enzyme that performs a key function in tumor cells. It is known that many copper complexes with bipyridine ligands that exhibit antitumor activity are effective inhibitors of topoisomerases, that is, key cell cycle enzymes. Recently, it was also shown in a number of works that \(5Z,9Z\)-dienoic acids are efficient nonspecific inhibitors of human topoisomerase.

In view of the foregoing, we hypothesized that copper carboxylates with pyridine ligands based on \(5Z,9Z\)-dienoic acids could also exhibit high cytotoxicity via targeted action on topoisomerases.

This communication presents preliminary results on the synthesis of copper bipyridine complexes based on \(5Z,9Z\)-eicosadienoic acid, which exhibited the highest inhibitory activity toward topoisomerase I, and study of its antitumor properties in vitro on several tumor cell lines of various etiology by means of flow cyttofluorometry.

2. RESULTS AND DISCUSSION

The complex \(\text{Cu(bpy)}_2(5,9\text{-eicd})\) (bpy is 2,2’-bipyridyl, 5,9-eicd is \(5Z,9Z\)-eicosadienoic acid) 1 was synthesized by the reaction of \(\text{Cu(OAc)}_2\cdot\text{H}_2\text{O}\) with 2,2’-bipyridine (2 equiv) and...
μ-plasmid DNA (pHOT1). (Figure 1B, samples 8−12) as an increase in the number of formed topoisomers (lanes 2−10) as the residual amount of the supercoiled plasmid DNA and inhibited by 2 nM the test compound) results in decrease in relaxation of supercoiled plasmid DNA with inhibition of topoisomerase I (Topogen) by the complex Cu(bpy)$_2$(5,9-eicd) (bpy is 2,2′-bipyridyl, arach: arachidic acid) 1 and Cu(bpy)$_2$(arach) (bpy: 2,2′-bipyridyl, arach: arachidic acid) 2 synthesized in this work to inhibit topoisomerase I.

We studied the ability of the compounds Cu(bpy)$_2$(5,9-eicd) (bpy: 2,2′-bipyridyl, 5,9-eicd: 5,9-eicosadienoic acid) 1 and Cu(bpy)$_2$(arach) (bpy: 2,2′-bipyridyl, arach: arachidic acid) 2, was prepared as a reference compound (Figure 1).

The results presented in Figure 2A,B indicate that the relaxation of supercoiled plasmid DNA with inhibition of topoisomerase I (Topogen) by the complex Cu(bpy)$_2$(5,9-eicd) (in this case, 3 enzyme units of topoisomerase I are inhibited by 2 nM the test compound) results in decrease in the residual amount of the supercoiled plasmid DNA and increase in the number of formed topoisomers (lanes 2−10) as the concentration of the test compound is increased from 1 to 100 nM. When the compound concentration is in the range from 1 to 20 nM, the supercoiled plasmid is predominantly accumulated upon addition of ethidium bromide to the gel (Figure 1B, samples 8−12), while at concentrations above 20 nM (Figure 2B, samples 3−5), the level of the open circular form increases. These results lead to the conclusion that Cu(bpy)$_2$(5,9-eicd) suppresses the topo I catalytic activity; however, its action is dose-dependent, and in concentrations of 20 nM and higher, it can also influence the formation of covalent complexes of DNA with topo I. Compound 2 did not show an inhibitory effect on the enzyme topoisomerase I. Thus, the synthesized copper complex has more than 10 times higher human topoisomerase inhibitory activity than the initial unsaturated acid.

The quantitative and qualitative analyses of cell viability, cell cycle, and apoptosis-inducing activity of Cu(bpy)$_2$(5,9-eicd) (bpy is 2,2′-bipyridyl, 5,9-eicd is 5,9-eicosadienoic acid) were performed by means of Guava Nexin Reagent, Guava Cell Cycle, and Guava ViaCount kits (Millipore).

The cytotoxic activity of the complex Cu(bpy)$_2$(5,9-eicd) (bpy is 2,2′-bipyridyl, 5,9-eicd is 5,9-eicosadienoic acid) in vitro against the Jurkat, HL-60, K562, and U937 human leukemia cells and HEK293 kidney cancer cells was tested using the Guava ViaCount kit (Millipore) (Table 1).

The complex Cu(bpy)$_2$(5,9-eicd) in concentrations from 0.05 to 0.22 μM exhibited a clearly pronounced cytotoxic effect against all types of cancer cells used in the tests. However, the highest CC$_{50}$ value was found for the A2780cis cell line (0.47 μM), whereas the CC$_{50}$ values for Jurkat, K562, HL-60, and U937 human leukemia cells and HEK293 kidney cancer cells were 0.05, 0.1, and 0.08 μM, respectively. It is noteworthy that the arachidic acid-based copper complex 2 and cisplatin taken as reference compounds showed substantially lower antitumor activities in vitro than complex 1 (Table 1).

It should be noted that the synthesized compounds exhibit good selectivity index (SI) (SI = CC$_{50}$ fibroblasts/CC$_{50}$ cancer

Table 1. Inhibition of Jurkat, HL-60, K562, U937, A2780cis, A2780, and HEK293 Cell Viability by the Complexes Cu(bpy)$_2$(5,9-eicd) (1) and Cu(bpy)$_2$(arach) (2), CC$_{50}$ (μM) ± SE (μM)

| Compound | Jurkat | HL-60 | K562 | U937 |
|----------|--------|-------|------|------|
| CC$_{50}$ (1) | 0.05 ± 0.001 | 0.04 ± 0.001 | 0.10 ± 0.007 | 0.08 ± 0.006 |
| CC$_{50}$ (2) | 11.26 ± 1.09 | 9.38 ± 0.91 | 15.28 ± 1.43 | 13.17 ± 1.29 |
| CC$_{50}$ (cisplatin) | 0.12 ± 0.004 | 0.10 ± 0.003 | 0.21 ± 0.006 | 0.18 ± 0.006 |
| HEK293 | 0.22 ± 0.008 | 0.47 ± 0.006 | 0.23 ± 0.004 | 1.07 ± 0.018 |
| A2780cis | 18.47 ± 1.82 | 26.89 ± 2.14 | 22.11 ± 2.24 | 76.32 ± 2.44 |
| A2780 | 0.32 ± 0.015 | 0.94 ± 0.028 | 0.35 ± 0.006 | 2.26 ± 0.029 |
| Fibrobl. | 0.05 ± 0.003 | 0.21 ± 0.006 | 0.10 ± 0.006 | 0.08 ± 0.006 |

SZ9Z-eicosadienoic acid (1 equiv) in methanol by a modified procedure (Figure 1). A similar copper complex based on arachidic acid, Cu(bpy)$_2$(arach) (bpy is 2,2′-bipyridyl, arach is arachidic acid) 2, was prepared as a reference compound (Figure 1).
cells); in particular for compound 1, the selectivity index varies from 2 to 18 with respect to all tumor cells.

The Cu(bpy)$_2$(5,9-eicd)-induced apoptosis in the Jurkat, HL-60, K562, U937, and HEK293 cell cultures was estimated by the detection of phosphatidylserine externalization on the plasmatic membrane after treatment of cell cultures with the test compound. It is worth noting that the effect of the complex Cu(bpy)$_2$(5,9-eicd) on the induction of apoptosis in Jurkat cells is more pronounced than in other types of cells, which is in line with the higher cytotoxicity of this compound against this cell line. As can be seen from Figure 3, the action of Cu(bpy)$_2$(5,9-eicd) on the HEK293 tumor cell culture induces a substantial dose-dependent increase in the number of apoptotic cells occurring at early and late apoptosis stages.

Figure 3. HEK293 cells treated with different concentrations of complex Cu(bpy)$_2$(5,9-eicd) were double-stained with annexine V/PI and analyzed by flow cytometry. (A) Control; (B) 1 (0.025 μM); (C) 1 (0.05 μM); and (D) 1 (0.1 μM).

Figure 4. Conventional light microscopy of HEK293 cells treated with different concentrations of the test compound after 24 h of exposure. (A) Control; (B) 1 (0.025 μM); (C) 1 (0.05 μM); and (D) 1 (0.1 μM).

Figure 5. Results of cell-cycle analysis by flow cytometry and representative of the profiles of cell cycle distribution in three independent experiments is shown. Jurkat cells treated with complex Cu(bpy)$_2$(5,9-eicd) for (A) absence of complex, (B) 24 h, (C) 48 h, and (D) 72 h. The percentage of Jurkat cells after staining with propidium iodide. Data are presented as mean ± standard deviation of three independent experiments.
The highest percentage of early and late apoptosis (~91%) was observed at a compound concentration of 0.1 μM. In particular, as shown in Figure 3, both early and late apoptosis stages for the Jurkat cells increase as compared with HL60 (p \leq 0.0003), U937 (p \leq 0.0005), HEK293 (p \leq 0.0007), and K562 (p \leq 0.00019).

Under conventional light microscopic examination, HEK293 cells treated with the complex Cu(bpy)\textsubscript{2}(5,9-eicd) differed from the control untreated sample by the presence of clear-cut morphological changes. The complex Cu(bpy)\textsubscript{2}(5,9-eicd) induced nuclear condensation and apoptotic body formation. These morphological changes, characteristic of early apoptosis, were visible as soon as 3 h after treatment. Figure 4 shows cells with different concentrations of the test compound after 24 h of exposure.

To find out whether the retarding effect exerted by complex Cu(bpy)\textsubscript{2}(5,9-eicd) is caused by the cell cycle arrest, we studied the distribution of cell cycle phases for five cell lines, Jurkat, HL60, K562, and U937, and the adhesion cell culture HEK293 after the appropriate treatment with the test compound by flow cytometry. According to cell cycle studies by the Guava Cell Cycle Reagent, the complex Cu(bpy)\textsubscript{2}(5,9-eicd) proved to be a potent inducer of hypodiploid cell population (sub-G1 phase) in all five cell lines. As shown in Figure 5, incubation of Jurkat cells for 24, 48, or 72 h after treatment with the complex resulted in 14.37 ± 1.02, 19.35 ± 1.33, or 35.99 ± 2.38% of hypodiploid cells, respectively. The percentage of cells in the G1 phase decreased compared with the control from 67.57 to 16.39%, whereas the percentage of cells in the S-phase substantially increased (from 27.50 to 47.69%) in the samples incubated for 72 and 48 h. These results indicate that the complex Cu(bpy)\textsubscript{2}(5,9-eicd) arrests the cell cycle in the S phase depending on the time of incubation.

It was shown that the complex Cu(bpy)\textsubscript{2}(5,9-eicd) is absorbed rather rapidly by cancer cells and induces apoptosis phenomena as soon as after a 3 h incubation. The presence of a high cytotoxicity on a panel of ovary cancer.

Thus, the newly synthesized copper complex (bipy)\textsubscript{2}Cu(5,9-eicd) based on SZ92Z-eicosadienoic acid and 2,2′-bipyridine shows a high antitumor activity in vitro toward Hek293, K562, HL60, Jurkat, and U937 cell lines, being more efficient than the known anticancer drug cisplatin. Furthermore, (bipy)\textsubscript{2}Cu(5,9-eicd) was found to arrest the cell cycle in the S phase, depending on the incubation time, and to act as an efficient human topoisomerase I inhibitor, which can help to make assumptions on the possible mechanism of its antitumor activity.

3. CONCLUSIONS

Thus, the newly synthesized copper complex (bipy)\textsubscript{2}Cu(5,9-eicd) based on SZ92Z-eicosadienoic acid and 2,2′-bipyridine shows a high antitumor activity in vitro toward Hek293, K562, HL60, Jurkat, and U937 cell lines, being more efficient than the known anticancer drug cisplatin. Furthermore, (bipy)\textsubscript{2}Cu(5,9-eicd) was found to arrest the cell cycle in the S phase, depending on the incubation time, and to act as an efficient human topoisomerase I inhibitor, which can help to make assumptions on the possible mechanism of its antitumor activity.

4. EXPERIMENTAL SECTION

4.1. General (Instruments).

Chromatographic analysis was performed on a Shimadzu GC-9A instrument using a 2000 × 2 mm column, the SE-30 (5%) stationary phase on Chromaton N-AW-HMDS (0.125×0.160 mm), helium carrier gas (30 mL/min), and temperature programming from 50 to 300 °C at a 8 °C/min rate. IR spectra were recorded on a Bruker VERTEX 70V FTIR spectrometer using KBr disks over the range of 400–4000 cm\textsuperscript{-1}. Melting points were recorded on a Stuart SMP3 advanced digital melting point apparatus. The \textsuperscript{1}H- and \textsuperscript{13}C NMR spectra were recorded in CDCl\textsubscript{3} on a Bruker Avance-400 spectrometer (100.62 MHz for \textsuperscript{13}C, 400.00 MHz for \textsuperscript{1}H). High-resolution mass spectra (HRMS) were measured on a Bruker maXis instrument using electrospray ionization. \textsuperscript{1}H NMR spectra were recorded in DMSO-d\textsubscript{6}.

In experiments on selective collisional activation (CAD), activation energy was set at the maximum abundance of fragment peaks (see figures legend). A syringe injection was used for solutions in MeCN–H\textsubscript{2}O, 50/50 vol % (flow rate 3 mL/min). Nitrogen was applied as a dry gas; interface temperature was set at 180 °C. Elemental analysis of the samples was determined by elemental analyzer firm KarloErba, model 1106. Reactions with organometallic compounds were performed in a dry argon flow. The solvents were dried and distilled immediately prior to use. Commercially available Cu(OAc)\textsubscript{2}, 2,2′-bipyridine, and arachidic acid (Aldrich) were used.
used. SZ,9Z-Eicosadienoic acid was synthesized according previously published technique.6

4.2. Chemistry. 4.2.1. Synthesis of Copper Bipyridine Complexes 1, 2 Based on SZ,9Z-Eicosadienoic and Arachidic Acids (General Procedure). A methanol solution (7.5 mL) of 2,2′-bipyridine (0.3 mmol) was added to a solution of Cu(OAc)2·H2O (0.3 mmol) in CH3OH–H2O (15 mL, 3:1) with vigorous stirring. A methanol solution (10 mL) of acid (0.15 mmol) was then added and the mixture stirred for 30 min. The reaction solution was filtered and left to stand at room temperature the precipitate evaluated after partial evaporation of solvent was collected by filtration.

4.2.1.1. Complex Cu(bpy),(5,9-eicd) (1). Yield 30%; Dark blue amorphous powder. IR (KBr pellet, υ, cm−1): 3428, 3118, 3077, 3057, 3030, 2920, 2851, 1654, 1605, 1577, 1562, 1469, 1377, 1159, 1108, 1022, 972, 931, 758, 731, 662. HMRS: [M + H]+ found = 683.3437; [M + Na]+ found = 705.3961; [M + Na]+ found = 725.2904, C40H55CuN4O2 anal. calcd 686.3621.

4.2.1.2. Complex Cu(bpy),(5,9-arach) (2). Yield 48%; Blue powder, mp = 126–128 °C. IR (KBr pellet, υ, cm−1): 3433, 3114, 3079, 3058, 3039, 2920, 2851, 1601, 1576, 1562, 1467, 1377, 1162, 1108, 1018, 972, 930, 754, 734, 660. HMRS: [M + H]+ found = 683.3437; [M + Na]+ found = 705.3961; [M + Na]+ found = 721.2904, C40H51CuN4O2 anal. calcd 682.3308.

4.3. Biology. 4.3.1. Cell Culture. Cells (Jurkat, K562, U937) were purchased from Russian Cell Culture Collection (Institute of Cytology of the Russian Academy of Sciences) and cultured according to standard mammalian tissue culture protocols and sterile technique. Human cancer cell lines HL60, HEK293, A2780cis, and A2780 were obtained from the HPA Culture Collections (U.K.). All cell lines used in the study were characterized for mycoplasma and viral contamination.12

HEK293 and fibroblasts cell line was cultured as monolayers and maintained in Dulbecco’s modified Eagle’s medium (DMEM, Gibco BRL) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution at 37 °C in a humidified incubator under a 5% CO2 atmosphere.

Cells were maintained in the DMEM medium (adherent cell culture HEK293, A2780cis, A2780, and fibroblasts) or RPMI 1640 (Jurkat, K562, and U937) (Gibco) supplemented with 4 mM glutamine, 10% FBS (Sigma), and 100 units/mL penicillin-streptomycin (Sigma). HL60 cells were cultured in RPMI 1640 with 20% FBS. All types of cells were grown in an atmosphere of 5% CO2 at 37 °C. The cells were subcultured at 2–3 days intervals. Adherent cells HEK293, fibroblasts, and A2870cis were suspended using trypsin/ethylenediaminetetraacetic acid and counted after they have reached 80% confluency. Cells were then seeded in 24-well plates at 5 × 104 cells per well and incubated overnight. Jurkat, HL-60, K562, and U937 cells were subcultured at 2 day intervals with a seeding density of 1 × 105 cells per well for 4 days and 10 days. The samples were acquired on NovoCyte 2000 Flow Cytometry System (ACEA) equipped with 488 nm argon laser. Detection of 7-AAD emission was collected through a 675/30 nm filter in the FL4 channel.14

4.3.3. Viability and Apoptosis. Apoptosis was determined by flow cytometric analysis of annexin V and 7-aminoactinomycin D staining. Briefly, 200 μL of Guava Nexin reagent (Millipore, Bedford, MA) was added to 5 × 105 cells in 200 μL, and the cells were incubated with the reagent for 20 min at room temperature in the dark. At the end of incubation, the cells were analyzed on NovoCyte 2000 flow cytometry system (ACEA).15

4.3.4. Cell Cycle Analysis. Cell cycle was analyzed using the method of propidium iodide staining. Briefly, the cells were plated in 24-well round-bottom plates at a density 10 × 105 cells per well, centrifuged at 450g for 5 min, and fixed with ice-cold 70% ethanol for 24 h at 0 °C. Cells were then washed with PBS and incubated with 250 μL of Guava Cell Cycle Reagent (Millipore) for 30 min at room temperature in the dark. Samples were analyzed on NovoCyte 2000 flow cytometry system (ACEA).16

4.3.5. DNA Topoisomerase I Assay. 7-A2780cis cell culture Cells were seeded in two 8-well plates with an integrated microelectronic sensor array in 600 μL of culture medium (iTCelligence real-time cell analyzer; ACEA Biosciences, San Diego, CA). After 24 h, the drugs were added for a total volume of 100 μL. The cell proliferation and survival were monitored in real-time by measuring the cell to electrode responses of the seeded cells. In each individual E-well, the cell impedance was measured and converted to cell index (CI) values by the RTCA software version 1.2 (Roche Diagnostics GmbH). The graphs were generated in real time by the iTCelligence system. Untreated and DMSO treated cells served as controls.16

### AUTHOR INFORMATION

Corresponding Authors

*E-mail: Drzhemilev@mail.ru. Tel/Fax: +7(347)2842750 (L.U.D.).
*E-mail: DyakonovVA@gmail.com (V.A.D.).

ORCID

Vladimir A. Dyakonov: 0000-0002-7787-5054

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

### ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (Grant No. 18-29-09068) and Russian Science Foundation (Grant No. 14-13-00263). 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. The biological studies of Cu complexes were done in the Laboratory of Molecular Design and Drug Bioscreening at the Institute of Petrochemistry and Catalysis of RAS. HMRS data were recorded in the...
Cleavage Activity of Ferrocene-Conjugated Ternary Copper(II) Complexes. Organometallics 2009, 28, 1493−1505. (c) Maity, B.; Roy, M.; Banik, B.; Majumdar, R.; Dighe, R. R.; Chakravarty, A. R. Ferrocene-Promoted Photoactivated DNA Cleavage and Anticancer Activity of Terpyridyl Copper(II) Phenanthroline Complexes. Organometallics 2010, 29, 3632−3641. (d) Selvakumar, B.; Rajendiran, V.; Maheswari, P. U.; Stoeckli-Evans, H.; Palaniandavar, M. Structures, spectra, and DNA-binding properties of mixed lipid and copper(II) complexes of iminodiacetic acid: The novel role of dinucleo co-ligands on DNA conformation and hydrolytic and oxidative double strand DNA cleavage. J. Inorg. Biochem. 2006, 100, 316−330. (e) Gandin, V.; Porchia, M.; Tisato, F.; Zanella, A.; Severin, E.; Dolmella, A.; Marzano, C. Total Synthesis of the Teicoplanin Aglycon. J. Am. Chem. Soc. 2013, 56, 7416−7417. (f) Majouga, A. G.; Zvereva, M. I.; Rubtsova, M. P.; Skvortsov, D. A.; Mironov, A. V.; Azhibek, D. M.; Krasnovskaya, O. O.; Gerasimov, V. M.; Udina, A. V.; Vorozhtsov, N. I.; Belogazkina, E. K.; Agron, L.; Mikhail, L. V.; Tetryakova, A. V.; Zyk, N. V.; Zefirov, N. S.; Kabanov, A. V.; Dontsova, O. A. Mixed Valence Copper(III) Binuclear Complexes with Unexpected Structure: Synthesis, Biological Properties and Anticancer Activity. J. Med. Chem. 2014, 57, 6252−6257. (g) Paterson, B. M.; Donnelly, P. S. Copper complexes of bis(thiosemicarbazones): from chemotherapy agents to diagnostic and therapeutic radiopharmaceuticals. Chem. Soc. Rev. 2011, 40, 3005−3018. (h) Bica, L.; Meyerowitz, J.; Parker, S. J.; Caragounis, A.; Du, T.; Paterson, B. M.; Barnham, K. J.; Crouch, P. J.; White, A. R.; Donnelly, P. S. Cell cycle arrest in cultured neuroblastoma cells exposed to bis(thiosemicarbazonato) metal complexes. Biomol. 2011, 24, 117−133. (i) Chan-Stier, C. H.; Minkel, D.; Petering, D. H. Reactions of bis(thiosemicarbazone) copper(II) complexes with tumor cells and mitochondria. Bioinorg. Chem. 1976, 6, 203−217. (j) Naemoto, T.; Yoshino, G.; Ojika, M.; Sakagami, Y. Amphoteric acids and related long-chain fatty acids as DNA topoisomerase I inhibitors from an Australian sponge, Amphimedon sp.: Isolation, structure, synthesis, and biological evaluation. Tetrahedron 1997, 53, 16699−16710. (b) Carbbelle, N. M.; Betancourt, J. E.; Orellano, E. A.; Gonzalez, F. A. Total synthesis and biological evaluation of (5Z,9S)-5,9-hexadecadienoic acid, an inhibitor of human topoisomerase I. J. Nat. Prod. 2002, 65, 1715−1718. (a) Vassallo, O.; Castelli, S.; Biassoni, A.; Sengupta, S.; Das, P. K.; D’Annessa, I.; Otter, F.; Leoni, A.; Tagliatesta, P.; Majumder, H. K.; Desideri, A. Conjugated Eicosapentaenoic Acid (EPA) Inhibits L. donovani Topoisomerase I and has an Antiproliferative Effect on L. donovani Promastigotes. Open Antimicrob. Agents J. 2011, 3, 23−29. (d) Suzuki, K.; Shono, F.; Kai, H.; Uno, T.; Uyeda, M. Inhibition of topoisomerases by fatty acids. J. Enzym. Inhib. 2000, 15, 357−366. (e) Castelli, S.; Campagna, A.; Vassallo, O.; Tesauro, C.; Fiorani, P.; Tagliatesta, P.; Otter, F.; Falconi, M.; Majumder, H. K.; Desideri, P. Conjugated eicosapentaenoic acid inhibits human topoisomerase IB with a mechanism different from camptothecin. Arch. Biochem. Biophys. 2009, 486, 103−110. (f) Miyashita, Y.; Tsuzuki, T.; Etsuka, T.; Miyazawa, T.; Kobayashi, K.; Iwash, H.; Kuriyama, I.; Yonezawa, Y.; Takemura, M.; Yoshida, H.; Sakaguchi, K. Inhibitory action of 2,2′,6,6′-tetramethylpiperidine N-oxyl on topoisomerase I/II. J. Enzym. Inhib. 2007, 22, 369−374. (g) Ramakrishnan, S.; Rajendiran, V.; Palaniandavar, M.; Stoeckli-Evans, H.; Periasamy, V. S.; Akbarsha, M. A.; Srinag, B. S.; Krishnamurthy, H.; et al. Mixed-Ligand Copper(II)-phenolate Complexes: Effect of Coligand on Enhanced DNA and Protein Binding, DNA Cleavage, and Anticancer Activity. Inorg. Chem. 2007, 46, 8208−8221. (i) Ramakrishnan, S.; Shakhtipriya, D.; Suresh, E.; Periasamy, V. S.; Akbarsha, M. A.; Palaniandavar, M. Electronic and Steric Parameters of 76 N-Heterocyclic Carbene in Ni(CO)3(NHC). Inorg. Chem. 2011, 50, 6458−6461. (j) Barve, A.; Kumbhar, A.; Bhat, M.; Joshi, B.; Butcher, R.; Sonawane, U.; Joshi, R. Mixed-Ligand Copper(II) Maltolate Complexes: Synthesis, Characterization, DNA Binding and Cleavage, and Cytotoxicity. Inorg. Chem. 2009, 48, 9120−9132. (k) Ganeshpandian, M.; Loganathan, R.; Ramakrishnan, S.; Riyasdeen, A.; Akbarsha, M. A.; Palaniandavar, M. Interaction of mixed ligand copper(II) complexes with CT DNA and BSA: Effect of primary ligand hydrophobicity on DNA and protein binding and cleavage and anticancer activities. Polyhedron 2013, 52, 924−938. (l) Ganeshpandian, M.; Loganathan, R.; Ramakrishnan, S.; Riyasdeen, A.; Akbarsha, M. A.; Palaniandavar, M. Interaction of mixed ligand copper(II) complexes with CT DNA and BSA: Effect of primary ligand hydrophobicity on DNA and protein binding and cleavage and anticancer activities. Inorg. Chem. 2009, 48, 1309−1322. (m) Maity, B.; Roy, M.; Saha, S.; Chakravarty, A. R. Photoinduced DNA and Protein
(10) (a) Du, M.; Cai, H.; Zhao, X.-J. A Two-Dimensional Hydrogen-Bonding Supramolecular Architecture of bis-(2,2′-bi-
pyridine)[(p-phenyl-enedi-oxy)-diacetato]-copper(II) Tetrahydrate. Acta Crystallogr., Sect. E: Struct. Rep. Online 2004, 60, m1139–
m1141. (b) Boonmak, J.; Youngme, S.; Chotkhun, T.; Engkagul, C.; Chaiwit, N.; Albada, G. A.; Reedijk, J. Polynuclear Copper(II)
Carboxylates with 2,2′-Bipyridine or 1,10-Phenanthroline: Synthesis, Characterization, X-Ray Structures and Magnetism. Inorg. Chem.
Commun. 2008, 11, 1231–1235.
(11) Belyakov, P. A.; Kadentsev, V. I.; Chizhov, A. O.; Kolotyrkina, N. G.; Shashkov, A. S.; Ananikov, V. P. Mechanistic Insight into
Organic and Catalytic Reactions by Joint Studies Using Mass Spectrometry and NMR Spectroscopy. Mendeleev Commun. 2010,
20, 125–131.
(12) D’yakonov, V. A.; Tuktarova, R. A.; Dzhemileva, L. U.; Ishmukhametova, S. R.; Yunusbaeva, M. M.; Dzhemilev, U. M.
Catalytic cyclometallation in steroid chemistry V: Synthesis of hybrid molecules based on steroid oximes and (SZ,9Z)-tetradeca-5,9-
dienedioic acid as potential anticancer agents. Steroids 2018, 138, 14–20.
(13) D’yakonov, V. A.; Tuktarova, R. A.; Dzhemileva, L. U.; Ishmukhametova, S. R.; Yunusbaeva, M. M.; Dzhemilev, U. M.
Catalytic cyclometallation in steroid chemistry VI: Targeted synthesis of hybrid molecules based on steroids and tetradeca-SZ,9Z-diene-
1,14-dicarboxylic acid and study of their antitumor activity. Steroids 2018, 138, 6–13.
(14) D’yakonov, V. A.; Kadikova, G. N.; Dzhemileva, L. U.; Gazizullina, G. F.; Yunusbaeva, M. M.; Dzhemilev, U. M. Oxidative
skeletal rearrangement of bicyclo[4.2.2]deca-2,4,7,9-tetraenes to bicyclo[4.3.1]deca-2,4,8-triene-7,10-diols and study of the antitumor
activity of the products in vitro. Tetrahedron 2018, 74, 4071–4077.
(15) Dzhemileva, L. U.; D’yakonov, V. A.; Makarov, A. A.; Andreev, E. N.; Yunusbaeva, M. M.; Dzhemilev, U. M. The first total synthesis
of the marine acetylenic alcohol, lembelvane B — a selective inducer of early apoptosis in leukemia cancer cells. Org. Biomol.
Chem. 2017, 15, 470–476.
(16) Harati, K.; Behr, B.; Wallner, C.; Daigeler, A.; Hirsch, T.; Jacobsen, F.; Renner, M.; Harati, A.; Lehnhardt, M.; Becerikli, M.
Antiproliferative activity of epigallocatechin3gallate and silibinin on soft tissue sarcoma cells. Mol. Med. Rep. 2017, 15, 103–110.