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DNA binding and antitumor activities of platinum(IV) and zinc(II) complexes with some S-alkyl derivatives of thiosalicylic acid

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Abstract

A series of complexes of platinum(IV) (**C1**–**C5**) and zinc(II) (**C6**–**C10**) with S-alkyl derivatives of thiosalicylic acid were prepared and characterized. The interactions of the complexes with calf thymus DNA were analyzed by absorption (UV–Vis) and emission spectral studies (ethidium bromide displacement studies). The cytotoxic activities of complexes **C1**–**C10** were determined against mouse B cell lymphocytic leukemia cells (BCL1), human B-prolymphocytic leukemia (JVM-13), mouse mammary carcinoma cells (4T1), and human mammary carcinoma cells (MDA-MB-468) and compared to the activities of the free ligand precursors and cisplatin. The cytotoxicities of the platinum(IV) and zinc(II) complexes toward mouse tumor cell lines were higher compared with their effects on human tumor cell lines. The zinc(II) complex **C9** showed the highest antitumor activity toward the tested human cell lines, while the platinum(IV) complex C4 exhibited the highest antitumor activity toward mouse BCL1 and 4T1 cells. Both **C4** and **C9** have ligands derived from S-propyl thiosalicylic acid.

Introduction

Platinum(II) complexes present a valuable starting point in the development of metal complex-based anticancer therapy. The original such platinum(II) complex, cisplatin, exhibits limited activity against several tumors, including colon and breast cancer, and is frequently accompanied with the development of resistance [1]. Furthermore, cisplatin therapy is accompanied by severe side effects such as potentially fatal renal failure, bone marrow suppression, severe nausea, and immunodeficiency [2]. Problems associated with cisplatin application, including high reactivity and thus poor biological stability [3, 4], have resulted in the synthesis of new transition metal complexes with bioactive ligands [5–8]. Platinum(IV) complexes have greater inertness compared with corresponding platinum(II) complexes, and undesirable side reactions with proteins in vivo can be avoided using platinum(IV) complexes [9]. The inertness of platinum(IV) gives rise to several advantages such as oral administration, reduced toxicity, and a decrease in the amount of the complex being lost or deactivated in the path to the target cell [10–12]. The reactivity of platinum(IV) complexes toward DNA is enhanced in the presence of a reducing agent such as ascorbic acid, glutathione, and proteins with sulfhydryl groups [13]. This

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suggests that platinum(IV) complexes are reduced by cellular components to form the platinum(II) analogs that bind to DNA [14–16].

The efforts of several research groups, which were initially focused on the evaluation of platinum-based anticancer drugs, have now shifted to non-platinum metal-based agents in order to minimize the side effects [17–19]. Based on the assumption that endogenous metals may be less toxic for normal cells, the investigation of zinc-based complexes has started only recently. The link between zinc deficiency and cancer has been established in human, animal, and cell culture studies [20, 21]. Zinc deficiency causes oxidative DNA damage [22], and chromosome breaks have been reported in zinc-deficient diet-fed animals [23]. During the past two decades, a number of zinc(II) complexes with anticancer activity have been reported, such as zinc(II) phthalocyanine tumor photosensitizers [24], and semicarbazone and thiosemicarbazone [25, 26] zinc(II) complexes.

Previously, copper(II) complexes with S-alkyl derivatives of thiosalicylic acid have been shown to exert potential antitumor activity and to interact with DNA [27–29]. Therefore, in order to investigate the possibility of even greater cytotoxicities, the central metal was changed and analogous platinum(IV) and zinc(II) complexes were synthetized [30, 31].

Herein, the interactions with DNA and cytotoxic activity against murine and human leukemia and breast carcinoma cells of five S-alkyl derivatives of thiosalicylic acid, together with five platinum(IV) and five zinc(II) complexes, are described.

Experimental

Materials and measurements

All chemicals were obtained commercially and used without further purification. Elemental microanalyses were obtained with a Vario III CHNOS Elemental Analyzer, Elemental Analysensysteme GmbH. For infrared spectra, a Perkin-Elmer FTIR 31725-X spectrophotometer and KBr pellet technique were employed. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini-200 NMR spectrometer using TMS in DMSO-d_6 as an internal reference, at 22 °C. Ethidium bromide (EB) and calf thymus DNA (CT-DNA) were obtained from Sigma-Aldrich. The stock solution of CT-DNA was prepared in 0.01 M phosphate-buffered saline (PBS) (Sigma-Aldrich) at pH = 7.4, which gave a ratio of UV absorbances at 260 and 280 nm (A_{260}/A_{280}) of ca. 1.8–1.9, indicating that the DNA was sufficiently free of proteins. The concentration of DNA solution was determined by UV absorbance at 260 nm ($\varepsilon = 6600\ M^{-1}\ cm^{-1}$) [32–34].

Synthesis of the S-alkyl thiosalicylic acids

The thioacid ligands benzyl-(**L1**), methyl-(**L2**), ethyl-(**L3**), propyl-(**L4**), and butyl-(**L5**) were prepared by alkylation of thiosalicylic acid using the corresponding alkyl halogenides in alkaline water–ethanol solution. Thiosalicylic acid (1 mmol) was added to a 30% solution of ethanol in water (50 mL) and stirred. A solution of NaOH (2 mmol) in water (5 mL) was added to the acid suspension, whereupon the solution became clear. The corresponding alkyl halogenide (2 mmol) was dissolved in ethanol (5 mL) and transferred to the stirred solution. The resulting mixture was kept overnight at 60 °C. Ethanol was then evaporated off by gentle heating. Dilute hydrochloric acid (2 mol/dm^3) was added to the resulting aqueous solution, whereupon the S-alkyl thiosalicylic acid was precipitated as a white powder. The product was filtered off, washed with plenty of distilled water, and dried under vacuum overnight. Yield was 85–95%.

S-benzyl-thiosalicylic acid (L1) M.p. 179–180 °C, white powder. *Anal*. Calc. for S-bz-thiosal = $C_{14}H_{12}O_2S$ (Mr = 244.24): C, 68.84; H, 4.95; S, 13.10. Found: C, 68.50; H, 4.82; S, 13.07%. IR (KBr, cm^{-1}): 3414w, 3061m, 2920m, 2648m, 2559m, 1674s, 1584w, 1562m, 1463m, 1412m, 1317m, 1272s, 1255s 1154w, 1062m, 1046m, 897m, 743m, 711w, 652w, 551w. ^{1}H NMR (200mHz, $CDCl_3$, δ ppm): 4.17 (s, 2H, CH_2), 7.21–8.14 (m, 9H, Ar and bz). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 35.9 (CH_2), 124.1, 125.9, 126.7, 127.3, 127.9, 128.3, 128.6, 129.3, 131.0, 132.4, 136.8, 141.3 (Ar and bz), 167.5 (COOH).

S-methyl-thiosalicylic acid (L2) M.p. 165–166 °C, white powder. *Anal*. Calc. for S-met-thiosal = $C_8H_8O_2S$ (Mr = 168.144): C, 57.14; H, 4.80; S, 19.03. Found: C, 57.72; H, 4.76; S, 19.50%. IR (KBr, cm^{-1}): 3446w, 3068m, 2916m, 2652m, 2560m, 1674s, 1586w, 1561m, 1466m, 1412m, 1308m, 1291s, 1270s, 1255s, 1151w, 1062m, 1048m, 892m, 743m, 699w, 652w, 556w. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 2.48 (s, 3H, CH_3), 7.16–8.18 (m, 4H, Ar). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 15.6 (CH_3), 123.5, 124.4, 125.4, 132.5, 133.6, 144.4 (Ar), 171.6 (COOH).

S-ethyl-thiosalicylic acid (L3) M.p. 133–134 C, white powder. *Anal*. Calc. S-et-thiosal = $C_9H_{10}O_2S$ (Mr = 182.17): C, 59.33; H, 5.53; S, 17.57. Found: C, 59.21; H, 5.49; S, 17.68%. IR (KBr, cm^{-1}): 3435w, 3066m, 2972m, 2652m, 2562m, 1682s, 1588w, 1563m, 1466m, 1414m, 1315m, 1275s, 1252s, 1152w, 1063m, 1049m, 884m, 740m, 704w, 690w, 651w, 550w. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 1.42 (t, 3H, CH_3), 2.97 (q, 2H, CH_2), 7.16–8.17 (m, 4H, Ar). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 13.1 (CH_3), 26.2

(CH_2), 124.0, 125.9, 126.4, 132.6, 133.2, 142.9 (Ar), 171.4 (COOH).

S-propyl-thiosalicylic acid (L4) M.p. 104 °C, white powder. *Anal*. Calc. for S-pr-thiosal = $C_{10}H_{12}O_2S$ (Mr = 196.196): C, 61.21; H, 6.16; S, 16.31. Found: C, 60.18; H, 6.50; S, 16.18%. IR (KBr, cm^{-1}): 3414w, 3056m, 2979m, 2641m, 2555m, 1678s, 1588w, 1562m, 1462m, 1405m, 1310m, 1271s, 1257s, 1150w, 1062m, 1053m, 811m, 740m, 704w, 691w, 653w, 554w. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 1.1 (t, 3H, CH_3), 1.74 (m, 2H, CH_2), 2.92 (t, 2H, CH_2), 7.15–8.15 (m, 4H, Ar). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 13.8 (CH_3), 21.6 (CH_2), 34.1 (CH_2), 123.8, 125.6, 126.2, 132.5, 133.1, 143.1 (Ar), 171.6 (COOH).

S-butyl-thiosalicylic acid (L5) M.p. 82–83 °C, white powder. *Anal*. Calc. for S-bz-thiosal $C_{11}H_{14}O_2S$ (Mr = 210.222): C, 62.84; H, 6.71; S, 15.22. Found: C, 62.66; H, 6.81; S, 15.30%. IR (KBr, cm^{-1}): 3420w, 2955m, 2869m, 2641m, 2556m, 1674s, 1586w, 1560m, 1462m, 1408m, 1320m, 1270s, 1250s, 1153w, 1060m, 1048m, 924m, 810m, 738m, 704w, 651w, 553w. ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 0.96 (t, 3H, CH_3), 1.46 (m, 2H, CH_2), 1.78 (m, 2H, CH_2), 2.94 (t, 2H, CH_2), 7.15-8.16 (m, 4H, Ar). ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 13.7 (CH_3), 22.3 (CH_2), 30.2 (CH_2), 31.9 (CH_2), 123.8, 125.7, 126.3, 132.5, 133.1, 143.1 (Ar), 171.4 (COOH).

Synthesis of the platinum(IV) complexes

Potassium hexachloroplatinate(IV) (0.1000 g, 0.2056 mmol) was dissolved in hot water (10 mL), and the appropriate thiosalicylic acid **L1**–**L5** was added. The resulting mixture was stirred for 2 h, and during this time, an aqueous solution of LiOH (0.0099 g, 0.4112 mmol) in water (10 mL) was introduced. The resulting yellow precipitate was filtered off, washed with water, and air-dried [30]

Platinum(IV) complex of S-benzyl derivative of thiosalicylic acid [$PtCl_2$(S-bz-thiosal)$_2$] (C1) Yield: 0.15 g (58%). *Anal*. Calc. for [$PtCl_2$(S-bz-thiosal)$_2$] = $PtC_{28}H_{22}O_4S_2Cl_2$ (Mr = = 752.59): C, 44.68; H, 2.95; S, 8.52. Found: C, 44.26; H, 2.88; S, 8.60%. IR (KBr, cm^{-1}): 3437m, 3062w, 3028w, 2924w, 1629s, 1561s, 1493w, 1463w, 1412w, 1318s, 1254m, 1142w, 1046w, 868w, 799w, 750m, 697m, 667w, 652w, 552w. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 4.01 (s, 4H, CH_2), 7.23–8.24 (m, 18H, Ar и bz). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 17 (CH_2), 124, 125.5, 127.1, 127.4, 127.7, 133.1, 133.9, 136.2 (Ar и bz), 169.1 (COO^-).

Platinum(IV) complex of S-methyl thiosalicylic acid [$PtCl_2$(S-met-thiosal)$_2$] (C2) Yield: 0.12 g (56%). *Anal*. Calc. for [$PtCl_2$(S-met-thiosal)$_2$] = $PtC_{16}H_{14}O_4S_2Cl_2$ (Mr = 600.40): C, 32.00; H, 2.35; S, 10.68. Found: C, 31.54; H, 2.59; S, 10.22%. IR (KBr, cm^{-1}): 3436m, 2923w, 2794w, 2439w, 1693s, 1581s, 1552s, 1469w, 1423m, 1361s, 1290s, 1274s, 1149w, 1116w, 1056w, 970m, 858w, 798w, 754s, 697w, 653w, 568w. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 2.47 (s, 6H, CH_3), 7.41–8.30 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 15.6 (CH_3), 125, 125.5, 126.4, 129.9, 134.1, 137.2 (Ar), 169.3 (COO^-).

Platinum(IV) complex of S-ethyl thiosalicylic acid [$PtCl_2$(S-et-thiosal)$_2$] (C3) Yield: 0.13 g (58%). *Anal.* Calc. for [$PtCl_2$(S-et-thiosal)$_2$] = $PtC_{18}H_{18}O_4S_2Cl_2$ (Mr = 828.46): C, 34.40; H, 2.89; S, 10.20. Found: C, 34.14; H, 2.71; S, 10.11%. IR (KBr, cm^{-1}): 3436s, 2521w, 1692m, 1634s, 1563w, 1437w, 1404w, 1274w, 1143w, 1122w, 1050w, 997w, 872w, 794w, 749w, 693w, 643w, 568w. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 1.27 (t, 6H, CH_3), 2.81 (q, 4H, CH_2), 7.42–8.28 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 12.9 (CH_3), 13.8 (CH_2), 125.1, 126.4, 126.6, 133.3, 133.8, 137.1 (Ar), 169.2 (COO^-).

Platinum(IV) complex of S-propyl thiosalicylic acid [$PtCl_2$(S-pr-thiosal)$_2$] (C4) Yield: 0.13 g (56%). *Anal.* Calc. for [$PtCl_2$(S-pr-thiosal)$_2$] = $PtC_{20}H_{22}O_4S_2Cl_2$ (Mr = 656.51): C, 36.59; H, 3.38; S, 9.77. Found: C, 36.17; H, 3.30; S, 9.61%. IR (KBr, cm^{-1}): 3444m, 3061w, 2963w, 2930w, 2873w, 2600w, 1706m, 1639s, 1586m, 1562m, 1461w, 1436w, 1416w, 1293s, 1253m, 1138w, 1091w, 1052w, 863w, 798w, 753m, 691w, 652w. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 0.90 (t, 6H, CH_3), 1.34 (m, 4H, CH_2), 2.75 (t, 4H, CH_2), 7.40–8.31 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 13.0 (CH_3), 23,2 (CH_2), 12 (CH_2), 125.3, 126.1, 126.4, 133.2, 133.9, 136.9 (Ar), 169.1 (COO^-).

Platinum(IV) complex of S-butyl thiosalicylic acid [$PtCl_2$(S-bu-thiosal)$_2$] (C5) Yield: 0.14 g (59%). *Anal*. Calc. for [$PtCl_2$(S-bu-thiosal)$_2$] = $PtC_{22}H_{26}O_4S_2Cl_2$ (Mr = 684.56): C, 38.60; H, 3.83; S, 9.37. Found: C, 38.35; H, 3.71; S, 9.28%. IR (KBr, cm^{-1}): 3437w, 3054w, 2956m, 2931m, 2869m, 2629w, 1673m, 1644s, 1635s, 1583m, 1561m, 1462m, 1433w, 1410w, 1318w, 1286m, 1250m, 1137w, 1100w, 1060w, 1049w, 916w, 863w, 754m, 738m, 698w, 652w, 551w. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 0.89 (t, 6H, CH_3), 1.43 (m, 4H, CH_2), 1.60 (m, 4H, CH_2), 2.73 (t, 4H, CH_2), 7.41–8.28 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 13.4 (CH_3), 21.5 (CH_2), 31(CH_2), 10 (CH_2), 124.9, 125.9, 126.5, 133.1, 134.2, 136.9 (Ar), 168.9 (COO^-).

Synthesis of the zinc(II)complexes

Zinc(II) chloride (0.1000 g, 0.7337 mmol) was dissolved in hot water (10 mL), and the required S-alkyl thiosalicylic

acid **L1**–**L5** was added. The reaction mixture was heated at 50 °C for 2 h, and during this period, a solution of LiOH (0.0352 g, 1.4674 mmol) in water (10 mL) was added in small portions. The solution was then filtered and evaporated to a small volume. The white precipitate of zinc(II) complex was separated by filtration, washed with water, and air-dried [31].

Zinc(II) complex of S-benzyl thiosalicylic acid [Zn(S-bz-thiosal)$_2$] (C6) Yield: 0.25 g (61%). *Anal*. Calc. for [Zn(S-bz-thiosal)$_2$] = $ZnC_{28}H_{22}O_4S_2$ (Mr = 551.978): C, 60.92; H, 4.02; S, 11.62. Found: C, 60.53; H, 3.94; S, 11.71%. IR (KBr, cm^{-1}): 3428m, 3059w, 2922w, 1598s, 1580s, 1437m, 1403s, 1282w, 1259w, 1063w, 1044w, 846w, 744m, 697w. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 4.03 (s, 4H, CH_2), 7.26–8.25 (m, 18H, Ar и bz). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 26 (CH_2), 125, 126.4, 126.8, 127.2, 127.3, 128.9, 133.5, 134.3, 137.7, 138.6 (Ar и bz), 169.2 (COO^-).

Zinc(II) complex of S-methyl thiosalicylic acid [Zn(S-met-thiosal)$_2$] (C7) Yield: 0.19 g (64%) *Anal.* Calc. for [Zn(S-met-thiosal)$_2$] = $ZnC_{16}H_{14}O_4S_2$ (Mr = 399.794): C, 48.06; H, 3.53; S, 16.04. Found: C, 47.69; H, 3.78; S, 15.71%. IR (KBr, cm^{-1}): 3436m, 2917w, 2859w, 1593s, 1576s, 1435m, 1399s, 1280w, 1256w, 1156w, 1065w, 953w, 847w, 744m, 654w. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 2.47 (s, 6H, CH_3), 7.42-8.30 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 15.6 (CH_3), 126, 126.3, 126.8, 133.4, 134.3, 137.2 (Ar), 169.4 (COO^-).

Zinc(II) complex of S-ethyl thiosalicylic acid [Zn(S-et-thiosal)$_2$] (C8) Yield: 0.18 g (58%) *Anal.* Calc. for [Zn(S-et-thiosal)$_2$] = $ZnC_{18}H_{18}O_4S_2$ (Mr = 427.846): C, 50.53; H, 4.24; S, 14.99. Found: C, 50.17; H, 4.07; S, 14.88%. IR (KBr, cm^{-1}): 3432m, 2954w, 2765w, 1595s, 1563s, 1436m, 1404s, 1273w, 1149w, 1122w, 1054w, 995w, 871w, 784w, 739m, 693w, 655w. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 1.27 (t, 6H, CH_3), 2.81 (q, 4H, CH_2), 7.43–8.27 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 13.9 (CH_3), 13 (CH_2), 125.1, 126.6, 126.5, 133.3, 134.2, 137.1 (Ar), 169.3 (COO^-).

Zinc(II) complex of S-propyl thiosalicylic acid [Zn(S-pr-thiosal)$_2$] (C9) Yield: 0.20 g (62%) *Anal.* Calc. for [Zn(S-pr-thiosal)$_2$] = $ZnC_{20}H_{22}O_4S_2$ (Mr = 455.898): C, 52.69; H, 4.86; S, 14.07. Found: C, 52.27; H, 4.78; S, 13.91%. IR (KBr, cm^{-1}): 3443m, 3061w, 2965w, 2931w, 2863w, 1591s, 1562s, 1462m, 1435m, 1312w, 1294w, 1252w, 1137w, 1092w, 1055w, 867w, 794w, 755m, 692w, 652w. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 0.90 (t, 6H, CH_3), 1.35 (m, 4H, CH_2), 2.77 (t, 4H, CH_2), 7.41–8.32 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 13.0 (CH_3), 24.2 (CH_2), 22 (CH_2), 125.3, 126.6, 126.5, 133.4, 134.2, 138.7 (Ar), 169.1 (COO^-).

Zinc(II) complex of S-butyl thiosalicylic acid [Zn(S-bu-thiosal)$_2$] (C10) Yield: 0.21 g (60%) *Anal*. Calc. for [Zn(S-bu-thiosal)$_2$] = $ZnC_{22}H_{26}O_4S_2$ (Mr = 483.950): C, 54.60; H, 5.42; S, 13.25. Found: C, 54.33; H, 5.19; S, 13.17%. IR (KBr, cm^{-1}): 3436m, 3053w, 2952w, 2934w, 2867w, 1614s, 1594s, 1582s, 1565m, 1467m, 1430m, 1408s, 1312s, 1291w, 1252w, 1139w, 1098w, 1063w, 1051w, 918w, 853w, 754m, 733m, 697w, 652w, 551w. ^{1}H NMR (200 MHz, DMSO-d_6, δ ppm): 0.88 (t, 6H, CH_3), 1.44 (m, 4H, CH_2), 1.60 (m, 4H, CH_2), 2.77 (t, 4H, CH_2), 7.42–8.28 (m, 8H, Ar). ^{13}C NMR (50 MHz, DMSO-d_6, δ ppm): 13.4 (CH_3), 21.6 (CH_2), 32 (CH_2), 20 (CH_2), 124.9, 126.6, 126.8, 133.3, 134.2, 138.5 (Ar), 168.8 (COO^-).

Absorption spectroscopic studies

The interactions of the complexes with CT-DNA were studied using UV–Vis spectroscopy at 310 K. Absorption titrations in buffer (0.01 M PBS, pH 7.4) were performed using a fixed complex concentration (8 µM) to which increments of the DNA stock solution were added (2.2 mM).

Fluorescence quenching measurements

EB-competitive studies for the complexes were carried out by fluorescence emission spectroscopy. DNA–EB was initially prepared by mixing 21 µM EB and 21 µM CT-DNA in 0.01 M PBS buffer solution (pH 7.4). The binding effect of the complexes was investigated step by step after the addition of a certain amount of complex solution into the solution of DNA–EB. The fluorescence intensities were measured with an excitation wavelength of 527 nm and detection of emission at 612 nm. Before measurements, each system was shaken and incubated at room temperature for 5 min.

Stock solutions for cytotoxicity studies

The complexes were dissolved in 10% DMSO in distilled water at a concentration of 10 mM and filtered through a 0.22-mm Millipore filter. These stock solutions were diluted in culture medium immediately before use. MTT, 3-(4,5–dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, was dissolved (5 mg/mL) in a phosphate-buffered saline having a pH of 7.2 and filtered through a 0.22-mm Millipore filter before use. All reagents were purchased from Sigma.

Cell culture

BCL1 (mouse B cell leukemia), 4T1 (mouse mammary carcinoma), JVM-13 (human B-prolymphocytic leukemia), and MDA-MB-468 (human mammary gland adenocarcinoma, metastatic) cells were purchased from the American Type Culture Collection (ATCC, Manassas, USA). The cells were maintained in DMEM (Sigma-Aldrich) or RPMI 1640 (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich), penicillin (100 IU/mL), and streptomycin (100 µg/mL) in a humidified atmosphere of 95% air/5% CO_2 at 37 °C. Subconfluent monolayers, in a log growth phase, were harvested by a brief treatment with 0.25% trypsin and 0.02% EDTA in phosphate-buffered saline (PBS, Sigma-Aldrich) and washed three times in serum-free PBS. The number of viable cells was determined by trypan blue exclusion assay.

Cell viability assay

The effects of the test compounds on cell viability were determined by MTT colorimetric technique [35]. The cells were diluted in growth medium to (5×10^4 cells/mL), and aliquots (5×10^3 cells/100 mL) were placed in individual wells in 96-well multiplates. The test compounds were serially diluted twofold in the medium to give concentrations raging from 1000 to 7.8 µM in growth medium and then added to wells with JVM-13 cells; the medium in the wells with adherent cells was exchanged the next day with 100 µL of fresh compound. Each compound was tested in triplicate. The cells were incubated at 37 °C in 5% CO_2 for 72 h. After incubation, the supernatant was removed and 15% MTT solution (5 mg/mL in PBS, 10 µL) in DMEM medium without FBS was added to each well. After an additional 4 h of incubation at 37 °C in 5% CO_2, the medium with MTT was removed and DMSO (150 µL) with glycine buffer (20 µL) was added to dissolve the crystals. The plates were shaken for 10 min. The optical density of each well was determined at 595 nm using a microplate Zenyth 3100 Multimode detector. The cytotoxicity was calculated using the formula:

$$\%\ \text{cytotoxicity} = 100 - ((E - B)/(S - B) * 100),$$

where B is the background of medium alone, S is the total viability/spontaneous death of untreated target cells, and E is the value for the experimental well.

LDH (lactic dehydrogenase) assay

Cytotoxicities of the test compounds were assayed using an In Vitro Toxicology Assay Kit (lactic dehydrogenase based) (Sigma-Aldrich). 4T1 cells were prepared and treated with the test compound in the same way as for the MTT assays. A high control, leading to 100% cytotoxicity by lysing the cells completely, was included in the assay. Cells exposed to medium only were used as a low control. After 24 h of treatment, the supernatant (50 µL) was transferred to a new multiplate and incubated with previously prepared substrate solution (100 µL). The plates were protected from light and incubated at room temperature for 30 min. The reaction was stopped by addition of stop solution, and the data were acquired by spectrophotometry at 490 nm. Experiments were repeated three times. The percentage of dead cells was calculated using the formula:

$$\%\ \text{dead cells} = (\text{exp. value} - \text{low control})/(\text{high control} - \text{low control}) \times 100.$$

Results and discussion

Synthesis and characterization

Thiosalicylate ligands **(L1–L5)** were synthesized by the reaction of thiosalicylic acid and the corresponding alkyl halides in alkaline water–ethanol solution [36]. The corresponding platinum(IV) complexes **(C1–C5)** were obtained by the direct reaction of K_2PtCl_6 with thiosalicylic acids **L1–L5** in water (Scheme 1) [30], while the zinc(II) complexes **(C6–C10)** were obtained by the direct reaction of $ZnCl_2$ with the thiosalicylic acids in water (Scheme 1) [31].

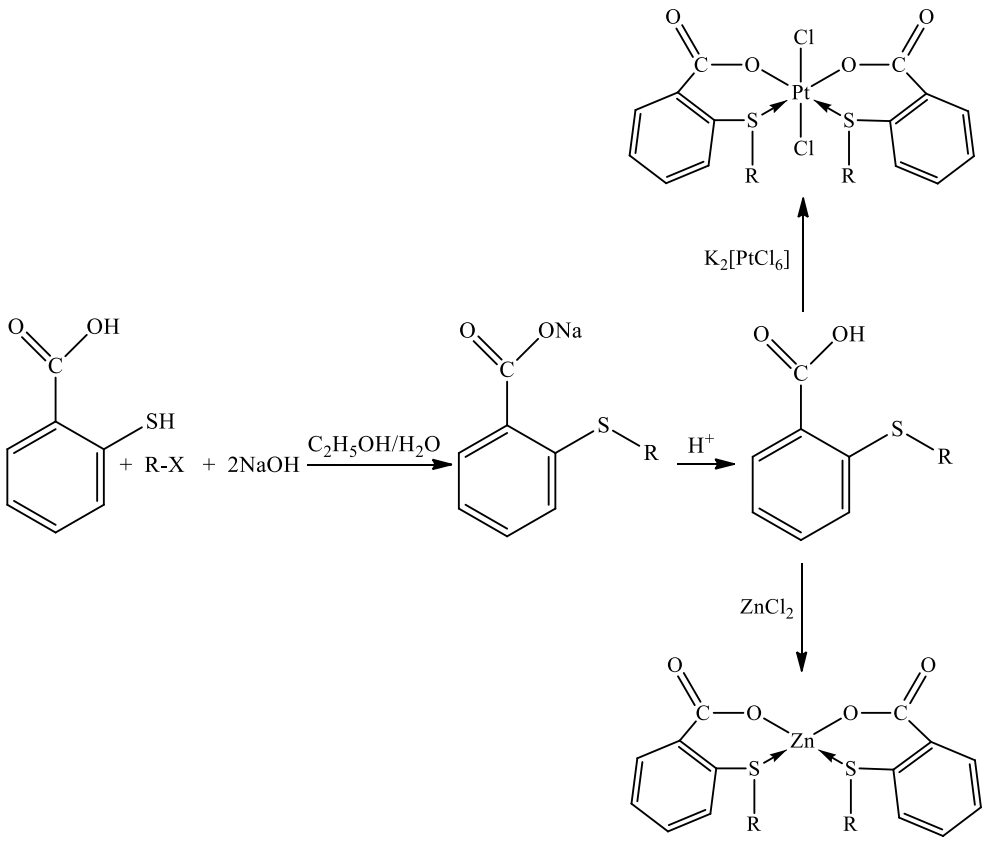
On the basis of microanalysis results, IR and NMR spectra of the proligands and the corresponding Pt(IV) and Zn(II) complexes, we conclude that the ligands are bidentately coordinated to the metal centers. However, we could not conclude anything about the exact geometries of the complexes. We were unable to obtain crystals of the complexes suitable for X-ray diffraction.

DNA binding studies

Absorption spectroscopy is one of the most universally employed methods for the determination of both the mode and extent of binding metal complexes with DNA. In general, transition metal complexes can bind to DNA via covalent (replacement of a labile ligand of the complex by a nitrogen base of DNA, e.g., guanine N7) and/or non-covalent (intercalation, electrostatic or groove binding) interactions [37]. The absorption intensity of a complex in the presence of DNA may either decrease (hypochromism) or increase (hyperchromism), with slight increase in the absorption wavelength (bathochromism). The absorption spectra of complex **C9** in the absence and in the presence of CT-DNA are shown in Fig. 1, and the spectra for the other complexes are presented in Fig. S1 of ESI.

The addition of CT-DNA to a solution of complex **C9** resulted in a significant hyperchromic effect with

Scheme 1 Preparation of the S-alkyl derivatives of thiosalicylic acid. R = benzyl (**L1**), methyl (**L2**), ethyl (**L3**), propyl (**L4**), butyl (**L5**), preparation of the platinum(IV) complexes **C1**–**C5** and the preparation of the zinc(II) complexes **C6**–**C10**



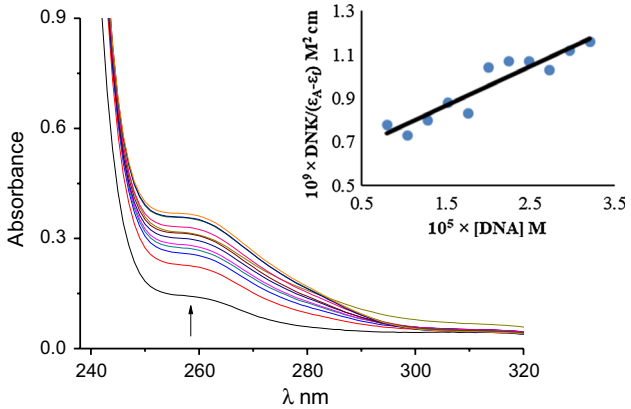


Fig. 1 Absorption spectra of complex **C9** in 0.01 M PBS (pH 7.4) upon addition of CT-DNA. [Zn complex] = 8×10^{-6} M, [DNA] = $(0.8–3.2)\times10^{-5}$ M. The arrow shows the change in absorbance upon increasing the DNA concentrations. Inset: Plot of [DNA]/($\varepsilon_A-\varepsilon_f$) versus [DNA]

the appearance of a new band at 262 nm, as shown in Fig. 1, and the spectrum of **C9** is shown in ESI, Fig. S4, $\lambda_{max} = 260$ nm. Considering that DNA also shows an absorption at 260 nm, this result confirms the presence of interactions as explained before. The behavior of the other complexes was quite similar upon addition of increasing amounts of CT-DNA (Fig. S1, ESI). A significant hyperchromic effect with the appearance of a new signal in the Uv–Vis spectra indicates an interaction between these complexes and CT-DNA [38].

Table 1 The DNA binding constants (K_b) and calculated Stern–Volmer constants (K_{sv}) for complexes **C1**–**C9**

Complex	K_b (M^{-1})	K_{SV} (M^{-1})
C1	$(1.13\pm0.01)\times10^4$	$(1.9\pm0.1)\times10^4$
C2	$(9.86\pm0.01)\times10^3$	$(7.4\pm0.1)\times10^3$
C3	$(2.50\pm0.02)\times10^4$	$(1.04\pm0.08)\times10^4$
C4	$(5.51\pm0.01)\times10^3$	$(6.12\pm0.09)\times10^3$
C5	$(2.22\pm0.02)\times10^4$	$(7.83\pm0.04)\times10^3$
C6	$(1.43\pm0.01)\times10^4$	$(2.50\pm0.05)\times10^3$
C7	$(1.18\pm0.02)\times10^4$	$(5.21\pm0.03)\times10^3$
C8	$(5.01\pm0.05)\times10^3$	$(1.13\pm0.02)\times10^3$
C9	$(3.33\pm0.02)\times10^4$	$(2.53\pm0.02)\times10^3$

The intrinsic binding constant of the complexes with CT-DNA (K_b) can be used to estimate the magnitude of binding strength. The values of K_b (M^{-1}) for all of the complexes calculated by equation (S1) are given in Table 1.

The high values for K_b confirm the binding of all of these complexes to CT-DNA. Complex **C9** exhibits the highest K_b value compared with the other Zn(II) complexes, while **C3** is the strongest binder among the Pt(II) complexes. In

comparison with the classical intercalator ethidium bromide (EB), for which $K_b = (1.23 \pm 0.07) \times 10^5$ M^{-1} [39], it can be seen that the present complexes exhibit lower binding constants.

The binding of the complexes to DNA was also investigated by fluorescence quenching. EB is a classical intercalator that gives intense fluorescence emission when it intercalates into the base pairs of DNA. Specifically, the maximum fluorescence emission is at 612 nm, when excited at 527 nm, due to the formation of an EB–DNA complex. If a test compound, which is also capable of DNA intercalation, is added to the EB–DNA complex, the fluorescence should decrease due to competitive intercalation.

The fluorescence quenching curves of EB bound to DNA in the absence and in the presence of the complexes are shown in Fig. 2 and Fig. S2, ESI. Increasing complex concentrations resulted in significant decreases in intensity of the emission band at 612 nm, indicating competition between the complexes and EB for DNA binding (Fig. 2 and Fig. S2).

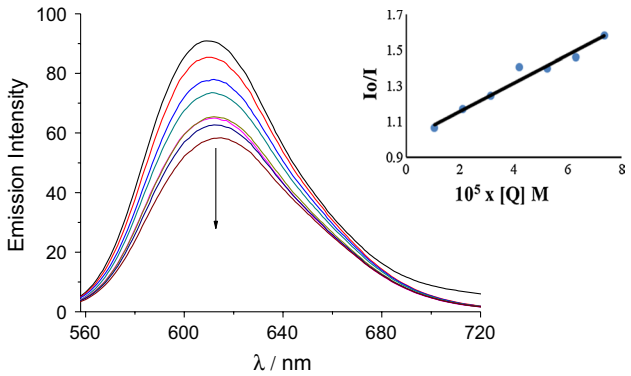


Fig. 2 Emission spectra of EB bound to DNA in the presence of complex **C5**. [EB] = 21 μM, [DNA] = 21 μM; [**C5**] = 0–73.5 μM; λ_{ex} = 527 nm. Arrows show the change in the intensity upon increasing the concentration of the complex. Inset: Stern–Volmer quenching plot of DNA–EB for complex **C5**

The Stern–Volmer plots for EB–DNA displacement (Fig. 2 and Fig. S2) confirm the fluorescence emission quenching. The Stern–Volmer quenching constants (K_{sv}) were calculated from the slopes of the plots of I_0/I versus [Q] ([Q] is the total concentration of quencher, Eq. (S2) ESI), inset Fig. 2 and Fig. S2) and are reported in Table 1. All of these complexes showed high quenching constants, indicating that they efficiently displace EB and bind to DNA. Complex **C7** exhibits the highest K_{SV} value in comparison with the other zinc(II) complexes, while **C1** exhibits the highest K_{SV} value in comparison with the other platinum(IV) complexes.

Antitumor activity

In order to investigate in vitro cytotoxic potential of the platinum(IV) and zinc(II) complexes, various concentrations of the complexes were applied to JVM-13 and pre-seeded BCL1, 4T1 and MDA-MB-468 cells, and the cell survival was determined after 24-h exposure by MTT assay. The results of these experiments, including cisplatin as a control substance, are presented in Table 2. All complexes showed a dose-dependent cytotoxic effect against all tested tumor cell lines (Fig. 3). Also these complexes, including cisplatin, had higher cytotoxic activities toward murine tumor cell lines (4T1 and BCL1) in comparison with human cell lines (MDA-MB-468 and JVM-13). Previously it was shown that copper(II) complexes of thiosalicylic acids had significantly lower cytotoxic activity toward murine and human colon carcinoma cell lines than cisplatin [28]. The free thiosalicylic acids had mild cytotoxicity only in the highest tested concentrations (500 and 1000 μM) (Fig. S3). It seems that coordination to a metal center greatly changes the antitumor activity of S-alkyl derivatives of thiosalicylic acid.

Table 2 IC_{50} values (μM) for 24 h of action of platinum(IV) and zinc(II) complexes and cisplatin on BCL1, 4T1, JVM-13, and MDA-468 cells, as determined by MTT assay

Compound	BCL1	4T1	JVM-13	MDA-468
C1	79.56 ± 12.11	38.81 ± 7.96	513.31 ± 87.74	305.66 ± 41.25
C2	65.83 ± 9.87	133.62 ± 19.62	433.29 ± 65.44	211.37 ± 31.41
C3	60.79 ± 8.11	56.13 ± 8.77	255.45 ± 18.78	187.53 ± 28.78
C4	2.47 ± 0.84	2.9 ± 1.01	88.78 ± 12.03	354.96 ± 52.17
C5	70.59 ± 11.24	42.88 ± 6.89	632.86 ± 87.96	252.91 ± 35.47
C6	109.08 ± 10.23	59.27 ± 10.21	115.06 ± 16.57	120.94 ± 15.71
C7	183.83 ± 24.55	85.31 ± 12.54	99.08 ± 14.74	125.86 ± 14.78
C8	187.53 ± 18.78	78.78 ± 10.12	224.40 ± 23.54	271.19 ± 24.96
C9	104.14 ± 14.11	49.41 ± 8.13	67.16 ± 10.02	72.73 ± 9.56
C10	2383.19 ± 121.26	7883.58 ± 251.14	416.35 ± 74.11	191.31 ± 21.11
Cisplatin	2.04 ± 0.79	3.44 ± 1.24	94.26 ± 9.89	27.38 ± 5.32

The data are presented as mean values ± SD (standard deviation) from three experiments

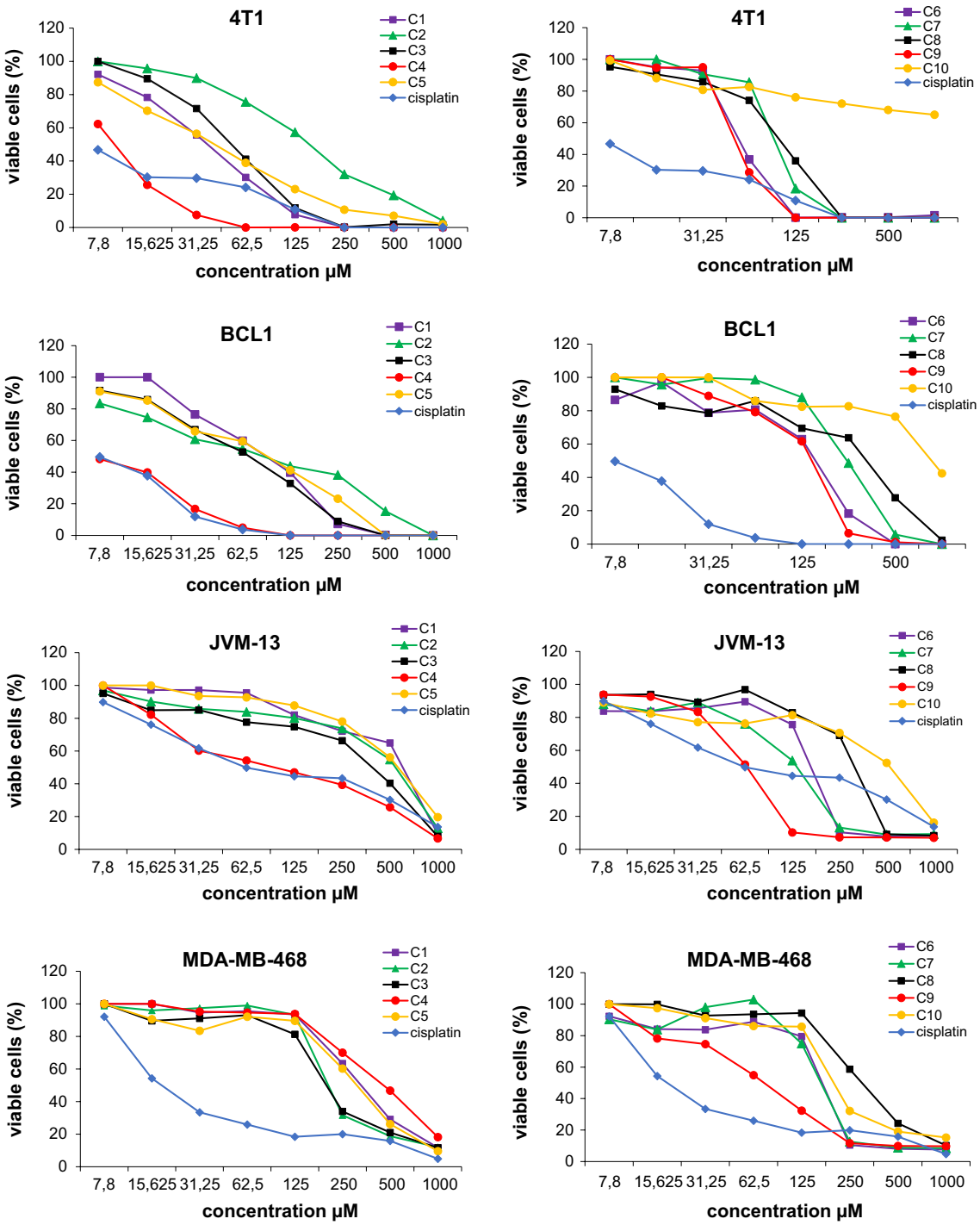


Fig. 3 Graphs of 4T1, BCL, MDA-MB-468, and JVM-13 cell survival after 24-h cell growth in the presence of the platinum(IV) and zinc(II) complexes. Mean values from three experiments are shown

Based on the obtained IC50 values, platinum(IV) complex **C4** had the highest cytotoxic potential toward murine cell lines. The cytotoxic potential of **C4** toward murine B cell leukemia cell line BCL-1 was similar to that of cisplatin (2.47 ± 0.84 versus 2.04 ± 0.79) and even higher toward murine mammary carcinoma cell line 4T1 (2.90 ± 1.01 versus 3.44 ± 1.24). These results are in accordance with previous findings, indicating high cytotoxicities of platinum(IV) complexes toward leukemia [40, 41] and breast cancer cell lines [42–44].

Among the zinc(II) complexes, propyl-S derivative **C9** had the highest activity toward murine cell lines 4T1 and

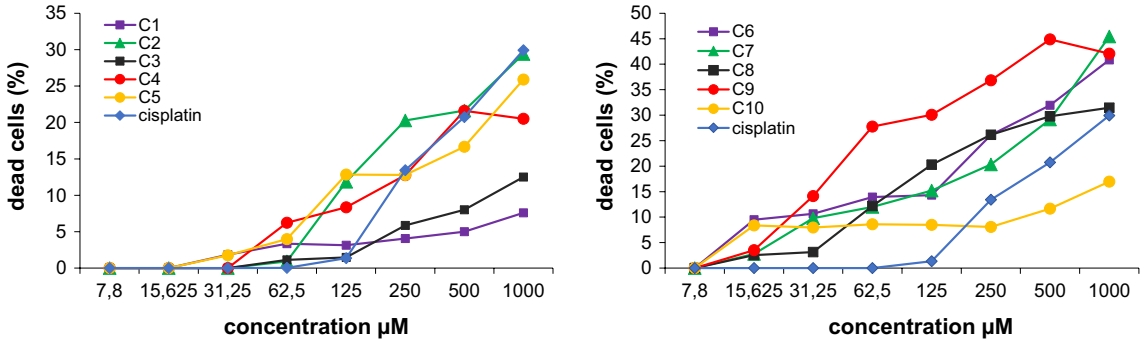


Fig. 4 LDH assay of 4T1 cells treated with platinum(IV) and zinc(II) complexes for 24 h. Mean values from three experiments are shown

BCL1. However, the activity of **C9** toward 4T1 and BCL1 was several times lower than that of cisplatin. The high cytotoxicity of platinum(IV) complex **C4** toward murine chronic lymphocytic leukemia cells is in agreement with previous studies [6, 40, 41]. The highest activity toward human tumor cell lines JVM-13 and MDA-MB-468 was shown by **C9**. Furthermore, the IC50 value for **C9** toward JVM-13 was lower than IC50 value for cisplatin (67.16 ± 10.02 versus 94.26 ± 9.89). High cytotoxicities of different zinc(II) complexes against different leukemia cells have been observed previously [45–47]. The S-propyl platinum(IV) complex **C4** also had higher cytotoxic activity toward JVM-13 than cisplatin. Importantly, the free propyl-S thiosalicylic acid had no cytotoxic potential toward tumor cell lines (Fig. S3), with very mild cytotoxicity only at a concentration of 1000 μM (cell viability approximately 80%).

The potential of these platinum(IV) and zinc(II) complexes to induce cell death was further investigated on 4T1 cells by LDH assay and found to follow a dose-dependent response (Fig. 4). Additionally, the zinc(II) complexes induced higher release of LDH from 4T1 cells in comparison with the platinum(IV) complexes. Platinum(IV) complexes **C1** and **C3** induced lower LDH release from 4T1 cells than cisplatin. On the other hand, all of the zinc(II) complexes in concentrations from 15.625 to 125 μM and **C6**–**C9** in all tested concentrations induced higher release of LDH in comparison with cisplatin (Fig. 4), indicating that these zinc complexes can induce necrotic cell death. Among the tested zinc(II) complexes, **C9** induced the highest release of LDH from 4T1 cells (Fig. 4).

Conclusion

Based on the observed results, it can be concluded that all of the studied complexes bind to CT-DNA. In particular, **C9** among the Zn(II) complexes and **C3** among the Pt(IV) complexes show the highest K_b values. Also these complexes showed high EB quenching constants, indicating that they can effectively displace EB from DNA. Among the zinc(II) complexes, **C7** showed the highest K_{sv} values, while among the platinum(IV) complexes, **C1** exhibited the highest K_{sv} values.

Both the platinum(IV) and zinc(II) complexes of these S-alkyl derivatives of thiosalicylic acid strongly interact with DNA. The platinum(IV) complexes exhibited the strongest antitumor activities toward murine cell lines, while the zinc(II) complexes showed the strongest cytotoxic activities toward human cell lines. Based on these results, the tumoricidal potential of S-propyl derivatives of thiosalicylic acid complexed with platinum(IV) and zinc(II) merits further investigation.

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