SYNTHESIS, CHARACTERIZATION, AND CYTOTOXICITY OF BINUCLEAR COPPER(II)-COMPLEXES WITH SOME S-ALKENYL DERIVATIVES OF THIOSALICYLIC ACID

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SAINTEA, KARAKTERIZACIJA I CITOTOKSIČNOST BINUKLEARNIH BAKAR(II)-KOMPLEKSA SA NEKIM S-ALKENIL DERIVATIMA TIOSALICILNE KISILENE

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ABSTRACT

New complexes of copper(II) with S-alkenyl derivatives of thiosalicylic acid (alkenyl = propenyl-(L1), isobutenyl-(L2)) have been synthesized and characterized by microanalysis, infrared spectra, magnetic measurements, and by NMR spectra. The cytotoxic activity of two newly synthesized precursor S-alkenyl derivatives of thiosalicylic acid were tested using an MTT colorimetric technique on HCT-116 human colon carcinoma cells. The cytotoxic effect of the copper(II)-complexes were higher compared to the cytotoxicity of the corresponding ligand (for concentrations from 31.25 to 250 μM). Copper(II)-complexes showed a slightly lower cytotoxicity compared to cisplatin. Complexes of copper(II) with S-alkenyl derivatives of thiosalicylic acid (at concentrations from 250 to 1000 μM) had a cytotoxic effect on HCT-116 cells compared to cisplatin.

Keywords: S-alkenyl derivatives of thiosalicylic acid, copper(II)-complexes, IR and NMR spectroscopy, cytotoxic activity

SAŽETAK


Ključne reči: S-alkenil derivati tiosalicilne kiseline, bakar(II)-kompleksi, IR i NMR spektroskopija, citoksična aktivnost

ABBREVIATIONS

MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
IR - infrared
NMR - nuclear magnetic resonance
Cu - copper
Zn - zinc
VD - Wilson’s disease
MD - Menkes disease
ROS - reactive oxygen species
Cu(NO3)2 - copper(II)-nitrate trihydrate
LiOH - lithium hydroxide
DMSO - dimethyl sulfoxide
DMEM - Dulbecco’s Modified Eagle Medium
FBS - fetal bovine serum
EDTA - ethylenediaminetetraacetic acid
PBS - phosphatebuffered saline
BM - bending magnet

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INTRODUCTION

After successful application of "cisplatin" (cis-diamminedichloroplatinum(II)) to chemotherapy in cancer patients, the exploration of other metal-complexes as new anticancer agents has continued, to enhance their specificity towards cancer cells, reduce toxic side effects, and prevent the development of resistance in human cancer cells (1-5).

Copper is an essential element for all organisms who live in environments rich in oxygen. This redox active metal converts oxidation state easily from a Cu(I) to Cu(II), and vice versa, as in chemical reactions and in physiological conditions (6). Copper is essential for the function of several enzymes and proteins, such as cytochrome oxidase, Zn, Cu-superoxide dismutase, lysyl oxidase, tyrosinase, and dopamine-b-monoxygenase (1, 6, 7).

The importance of copper is also reflected in the fact that its deficiency leads to congenital genetic diseases, such as Wilson’s disease (VD) and Menkes disease (MD) (8). Copper chelation therapy has attracted considerable attention for use in research and treatment of various neurodegenerative disorders (9).

The presence of proteins found in copper has been associated to metabolic changes in cancer cells. The exact role of copper in the treatment of cancer has not been sufficiently clarified (6, 10). It is assumed that copper is involved in the generation of ROS (reactive oxygen species) and the process of angiogenesis by stimulating the proliferation and migration of human endothelial cells (10-12).

Thiosalicylic acid and its derivatives have a wide range of applications. It has been used for the determination of metals (13, 14); as modifiers of graphite paste electrodes (15); as photoinitiators of free radical polymerization (16); in cosmetics (17); in the treatment of dermatological (18), inflammatory, allergic, and respiratory diseases (19); and as Ras-tumour growth inhibitors (20).

Copper(II)-complexes may exert cytotoxic activity on colon carcinoma cell lines. O’Halloran found that a copper complex (Cu[N-salicylidene-(glutamate)(H2O)4]·H2O) at concentrations of 50 and 100 μmol/L was shown to have distinct cytotoxic activity in human HT-29 colon cancer cells after 72 hours. Apoptosis was activated by the generation of large quantities of free radicals (21).

Various studies have confirmed the synthesis and structural characterization of different complexes of copper(II) with thiosalicylic acid as a ligand (22, 23). Complexes of copper(II) with the S-alkyl derivatives of thiosalicylic acid showed moderate antimicrobial activity and low antifungal activity (24). With consideration for these effects, new research examining the cytotoxic potential of copper(II) with S-alkyl derivatives of thiosalicylic acid has found that these derivatives exhibit a lower cytotoxicity compared to cisplatin in the human HCT-116 cell line and in murine cell lines CT26 and CT26.CL25 (25).

The first aim of our study was to synthesize two new ligands acting as S-alkenyl derivatives of thiosalicylic acid (alkenyl = propenyl-(L1), isobutenyl-(L2)), and two corresponding copper(II)-complexes with these ligands. The composition and structure of S-alkenyl derivatives of thiosalicylic acid was assumed on the basis of microanalysis, IR, and NMR spectroscopy. The composition and structure of synthesized complexes was confirmed based on the microanalysis, IR spectroscopy, and magnetic measurements. Another aim of our study was to investigate the cytotoxic potential of copper(II)-complexes with S-alkenyl derivatives of thiosalicylic acid on the human colon cancer cell line HCT-116.

MATERIALS AND METHODS

Materials and measurements

The reagents were obtained commercially and used without further purification. Elemental analyses were conducted on a Vario III CHNOS Elemental Analyser, Elemental Analysensysteme GmbH. For infrared spectra, a Perkin-Elmer FTIR 31725-X spectrophotometer, and KBr pellet technique were employed.

Syntheses

General procedure for the synthesis of S-alkenyl derivatives of thiosalicylic acid

The S-alkenyl derivatives of the thiosalicylic acid ligand (alkenyl = propenyl-(L1), isobutenyl-(L2)) were prepared (26) by alkylation of thiosalicylic acid using the corresponding alkenyl halides in alkaline water-ethanol solution.

S-propenyl derivative of thiosalicylic acid (S-propenylthio sal), (L1): M.p. 180-181°C, white powder, IR (KBr, cm-1): 3464, 3075, 2917, 2654, 2557, 1680, 1586, 1465, 1415, 1314, 1273, 1256, 1153, 1061, 1045, 889, 741, 701, 651, and 551. 1H NMR (200 MHz, CDCl 3, δ ppm): 3.44(t, 2H, CH2), 3.21 (d, 2H, CH2), and 7.42-8.30 (m, 4H, Ar). 13C NMR (50 MHz, CDCl 3, δ ppm): 38.2 (CH3), 115.9 (CH2), 136.5 (CH), 125.3; 125.6; 130.2; 131.7; 133.8; 140.1 (Ar), and 172.0 (COOH).

S-isobutenyl derivative of thiosalicylic acid (S-isobutenylthio sal), (L2): M.p. 182-183°C, white powder, IR (KBr, cm-1): 3445, 3076, 2967, 2647, 2556, 1676, 1585, 1562, 1463, 1412, 1317, 1272, 1253, 1154, 1061, 1046, 810, 743, 651, and 550. 1H NMR (200 MHz, CDCl 3, δ ppm): 1.82 (s, 3H, CH3), 4.98 (m, 2H, CH2), 3.44(t, 2H, CH2), and 7.42-8.30 (m, 4H, Ar). 13C NMR (50 MHz, CDCl 3, δ ppm): 22.5 (CH 3), 46.1 (CH2), 112.1 (CH), 125; 126.5; 126.7; 133.2; 134.1; 142.6 (Ar), and 168.1 (COOH).

Preparation of copper(II)-complex with S-prope nyl derivative of thiosalicylic acid [Cu(S-propenylthiosal)(H2O)4] (C1)

Copper(II)-nitrate trihydrate (0.1000 g, 0.4139 mmol) was dissolved in 10.0 mL of water on a steam bath, and S-propenyl derivative of thiosalicylate (0.1607 g, 0.8278
mmol) was subsequently added. The reaction mixture was heated for 3 h, and during this period 10.0 ml of LiOH wa-
ter solution (0.0199 g, 0.8278 mmol) was added in small ~
portions. The solution was filtered and evaporated to a small volume. The blue precipitate of copper(II)-complex was separated by filtration, washed with cold water, and air-dried. Yield: 0.2228 g (82.1%). Anal. Calc. for [Cu2(S-isobutenyl-thiosal)4(H2O)2] = Cu2C45H51O10S4 (Mr = 1007.232): C, 53.66; H, 5.10; and S, 12.73. Found: C, 53.47; H, 5.18; and S, 12.64. μ(294 K) = 1.86 μB.
IR (KBr, cm⁻¹): 3446, 2969, 2915, 1613, 1589, 1400, 1281, 1257, 1157, 1062, 847, 744, 719, 656, and 510.

Preparation of drug solutions

Complexes were dissolved in 10% dimethylsulfoxide (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 phos-
phate buffer saline having a pH of 7.2, and filtered through the 0.22 mm Millipore filter before use. All reagents were purchased from Sigma Chemicals.

Cell culture

HCT-116 cells were kindly provided by Dr Danijela Vignjević (Institute Curie, Paris, France). Cells were main-
tained in DMEM (Sigma Aldrich, Munich, Germany) supplemented with 10% fetal bovine serum (FBS, Sigma Aldrich, Munich, Germany), penicillin (100 IU/mL), and streptomycin (100 μg/mL) in a humidified atmosphere of 95% air and 5% CO2 at 37°C. Subconfluent monolayers in the logarithmic growth phase were harvested by a brief treatment with 0.25% trypsin and 0.02% EDTA in phos-
phate-buffered saline (PBS, PAA Laboratories GmbH) and washed three times in serum-free PBS. The number of vi-

Cytotoxicity assays

The effects of the tested compounds on cell viability were determined using the MTT colorimetric technique (27). HCT-116 cells were diluted with growth medium to 5·10⁴ cells/mL, and aliquots (5·10³ cells/100 mL) were placed in individual wells in 96-well plates. The next day the medium was exchanged with 100 μL of different com-

Preparation of copper(II)-complex with S-isobutene-

Synthesis and chemical characterization

S-alkenyl (R = propenyl-(L1), isobutenyl-(L2)) deriva-
tives of thiosalicylic acid were prepared (26) by alkylation of thiosalicylic acid using the corresponding alkenyl halo-
genides in an alkaline water-ethanol solution (Scheme 1). The corresponding complexes were obtained for cytotoxicity in three separate experiments.

RESULTS AND DISCUSSION

Synthesis and chemical characterization

S-alkenyl (R = propenyl-(L1), isobutenyl-(L2)) deriva-
tives of thiosalicylic acid were prepared (26) by alkylation of thiosalicylic acid using the corresponding alkenyl halo-
genides in an alkaline water-ethanol solution (Scheme 1). The corresponding complexes were obtained for cytotoxicity in three separate experiments.
We can conclude that there was a coordination of S-alkenyl derivatives of thiosalicylic acid with copper(II)-ions in forming binuclear complexes. Additionally, the strong sharp single symmetric stretching bands of the coordinated carboxyl groups of the S-alkenyl derivatives of thiosalicylic acid lie in the expected region (approximately 1400 cm⁻¹).

**Magnetic measurements**

Binuclear copper(II)-complexes (31, 32) are stable in dimeric form. The low value of μ eff at room temperature (approximately 1.86 BM) is indicative of an antiferromagnetic interaction between the two metal centres typical of binuclear carboxylates of copper(II) of the type: [Cu(R-COO)₂L]₂ (33–35). The main factor determining the magnitude of the antiferromagnetic interaction in the dimeric copper(II) carboxylates is the electronic structure of the bridging OCO moiety, as published previously (31, 32–35).

**Anticancer activity of copper(II)-complexes**

The MTT assay for cell viability shows that the two newly synthesized precursor S-alkenyl derivatives of thiosalicylic acid, as well as their corresponding copper(II)-complexes, exhibit cytotoxic activity in HCT-116 human colon carcinoma cells after 24 hours (Figure 1). The cytotoxic effect of the copper(II)-complexes was higher than the cytotoxicity of the corresponding ligands, especially for concentrations from 31.25 to 250 μM. Copper complexes showed slightly lower cytotoxicity compared to cisplatin. However, higher concentrations of C2 (250-1000 μM) had an almost equal cytotoxic effect on HCT-116 cells, relative to cisplatin.
CONCLUSION

The complexes of copper(II) with S-alkenyl derivatives of thiosalicylic acid (alkenyl = propenyl-(L1), isobutenyl-(L2)) have been synthesized and characterized by microanalysis, infrared spectroscopy, and magnetic measurements. The cytotoxic effect of the copper(II)-complex was higher compared to the cytotoxicity of the corresponding ligands. Copper(II)-complexes showed slightly lower cytotoxicity compared to cisplatin. Complexes with S-alkenyl derivatives of thiosalicylic acid in concentrations from 250 to 1000 μM had a cytotoxic effect on HCT-116 cells that was similar to cisplatin.

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