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Preliminary Investigations on Cytotoxic Activity of Four Nickel (II) Complexes with Mannich Type Ligands on Virus-Induced Tumor Cell Lines

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Cytotoxic activity of four nickel (II) complexes with ligands containing the antipyrine moiety N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) or N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) was evaluated on virus transformed cells. Two permanent cell lines were used in the experiments: LSCC-SF(Mc29), established from a transplantable chicken hepatoma induced by the myelocytomatosis virus Mc29 and LSR-SF(SR), derived from a transplantable sarcoma in rat induced by Rous sarcoma virus strain Schmidt-Ruppin (SR-RSV). The effect on cell viability was studied by neutral red uptake cytotoxicity assay. The results obtained showed that $Ni_2(BAMP)(CH_3COO)_4$ and $Ni_2(BAMP)(Cl)_4$ expressed time- and dose-dependent cytotoxic activity. $Ni(TAMEN)(ClO_4)_2$, $Ni(TAMEN)(NCS)_2$ as well as both ligands are no or very low toxic at the concentrations examined.

Key words: Mannich bases, pyrazolone, nickel, polynuclear complexes, cytotoxic activity, virus-transformed cells.

Introduction

In 1912 Mannich and Krösche discovered the property of formaldehyde to bind an amine with a carbon acid via a methylene bridge [13]. The method was utilized to obtain pharmaceutical products by implication of acids components which were recognized like substances with therapeutic action. Our main point of interest refers to study the Mannich base complexes of some first row metal ions, in order to explain their biological activity as well as to find new compounds with biological effects. In this frame, the Mannich bases N,N'-bis-(antipyryl-4-methyl- piperazine (BAMP) and N,N'-tetra-(antipyrylmethyl)-1,2-ethanediamine (TAMEN) as well as their metal complexes have been reported [7, 8, 9, 17, 21, 22]. The aim of the present study was to evaluate the influence of four newly synthesized nickel (II) complexes with BAMP or TAMEN on viability of

virus-transformed cells. Nickel is known to be an essential element for at least several animal species [5]. It has recently been reported that some Ni(II) complexes with different ligands express antineoplastic properties in vitro and in vivo [6, 10, 18, 19, 20].

Material and Methods

COMPOUNDS. Four Ni(II) complexes with ligands containing the antipyrine moiety N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) (Fig. 1) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) (Fig. 2) – $Ni(TAMEN)(ClO_4)_2$, $Ni(TAMEN)(NCS)_2$, $Ni_2(BAMP)(CHCOO_3)_4$, $Ni_2(BAMP)(Cl)_4$, as well as both ligands were investigated.

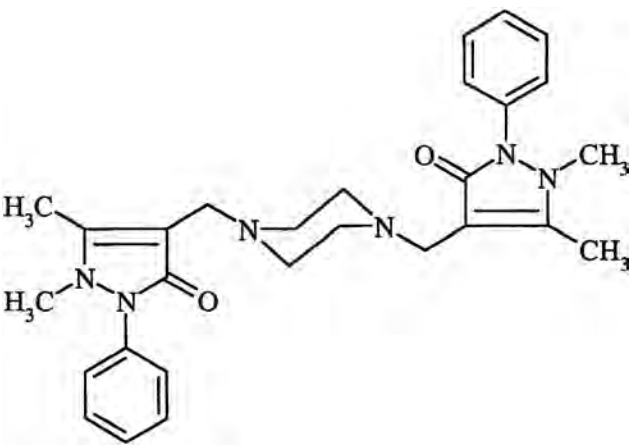

Fig. 1. N,N´-bis(4-antipyrylmethyl)-piperazine (BAMP)

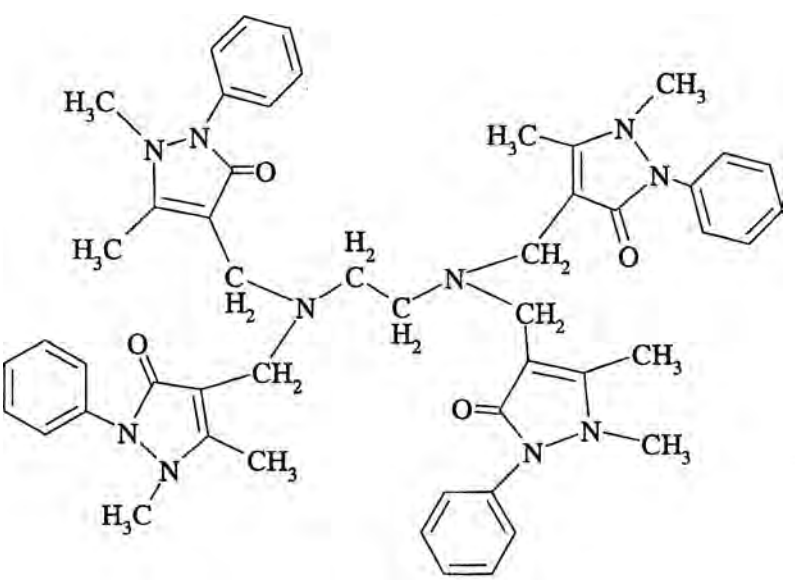

Fig. 2. N,N´-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN)

BAMP and **TAMEN** were obtained following a Mannich type condensation between antipyrine, piperazine or 1,2-ethandiamine and formaldehyde. The method was modified in order to obtain a pure compound.

$Ni_2(BAMP)Cl_4$. 0.972 g (2 mmol) BAMP dissolved in 25 cm^3 dioxan and 15 cm^3 ethanol at 60°C was treated with 0.475 g (2 mmol) $NiCl_2.6H_2O$ dissolved in 5 cm^3 water, under vigurous stirring. After two hours, a green compound was separated by filtration, washed with ethanol and dried on P_2O_5. Yield: 78 %.

$Ni_2(BAMP)(CH_3COO)_4$. 0.972 g (2 mmol) BAMP dissolved in 25 cm^3 methanol at 50°C was treated with 0.995 g (4 mmol) $Ni(CH_3COO)_2.4H_2O$ dissolved in 10 cm^3 ethanol. After two hours the light green suspension was concentrated, then was added ethyl ether. A light green compound was separated, washed with ethanol and dried on P_2O_5. Yield: 52 %.

$Ni(TAMEN)(ClO_4)_2$. 0,48 g (2 mmol) $NiCl_2 \cdot 6H_2O$ dissolved in 10 ml ethanol was mixed with 0,86 g (1 mmol) TAMEN dissolved in 15 ml ethanol at 45°C under stirring and then 1g (7 mmol, excess 75%) of $NaClO_4 \cdot H_2O$ dissolved in 8 ml ethanol was slowly poured. The blue mixture was further stirred for 30 minutes at 35 °C and for 30 minutes at room temperature. The resulting blue precipitate was filtered after 24 hours. Yield: 92 %

$Ni(TAMEN)(NCS)_2$, 0,24g (1 mmol) $NiCl_2 \cdot 6H_2O$ dissolved in 8 ml ethanol at 45 °C was treated with 0,43g (0,5 mmol) of TAMEN dissolved in 10 ml ethanol at 45°C. A solution of 0,26 g (3,5 mmol, excess 75%) NH_4SCN in 5ml ethanol was then added and the mixture was further stirred for an hour at room temperature. The microcrystalline green product was filtered on the next day and dried on $CaCl_2$. Yield: 77 %.

Nickel (II) complexes as well as their ligands were dissolved in dimethylsulfoxide (DMSO, Serva) and then diluted in culture medium.

Cell lines and cultivation. The following permanent cell lines were used in the experiments: LSCC-SF(Mc29), established from a transplantable chicken hepatoma induced by the myelocytomatosis virus Mc29 [2] and LSR-SF(SR), derived from a transplantable sarcoma in rat induced by Rous sarcoma virus strain Schmidt-Ruppin (SR-RSV) [1].

Cells were grown as monolayer cultures in a combination of medium H-199 and Minimum Essential medium (AppliChem, Germany), supplemented with 5–10% fetal bovine serum (Cambrex, Belgium), 100 U/ml penicillin and 100 μg/ml streptomycin. The cultures were maintained at 37°C in a humidified CO_2 incubator. For routine passages adherent cells were detached using a mixture of 0.05% trypsin (Gibco) – 0.02% ethylendiaminotetraacetic acid (EDTA). The experiments were performed during the exponential phase of cell growth.

Cytotoxicity assay. The cells were seeded in 96-well plates (Cellstar) at a concentration of 2×10^4 cells/well. At the 24th h cells from monolayers were washed and covered with media modified with different concentrations of the compounds tested (each concentration in 6 to 8 repetitions). Samples of cells grown in non-modified medium served as a control. After 24 h and 48 h incubation periods, each plate was examined under inverted microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. Effect of the compounds on cell viability was evaluated by neutral red cytotoxicity assay [3]. Relative cell viability, expressed as a percentage of the untreated control, was calculated for each concentration. All experiments were in triplicate.

Statistical analysis. The data are presented as mean ± standard error of the mean. Statistical differences between control and treated groups were assesses using one-way analysis of variance (ANOVA) followed by Dunnett post-hoc test.

Results and Discussion

Applied at concentrations of 1, 10, 50, 100 and 200 μg/ml $Ni(TAMEN)(ClO_4)_2$, $Ni(TAMEN)(NCS)_2$ as well as BAMP and TAMEN did not significantly reduce the viability of virus-transformed cells investigated. More than 94% of LSR-SF(SR) and > 90% of LSCC-SF(Mc29) cells cultivated in the presence of these compounds were found to be alive after 24 h and 48 h treatment. At the same time the other two metal complexes – $Ni_2(BAMP)(CH_3COO)_4$ and $Ni_2(BAMP)(Cl)_4$, expressed time and dose-dependent cytotoxic activity against both cell lines used in the experiments (Fig. 3).

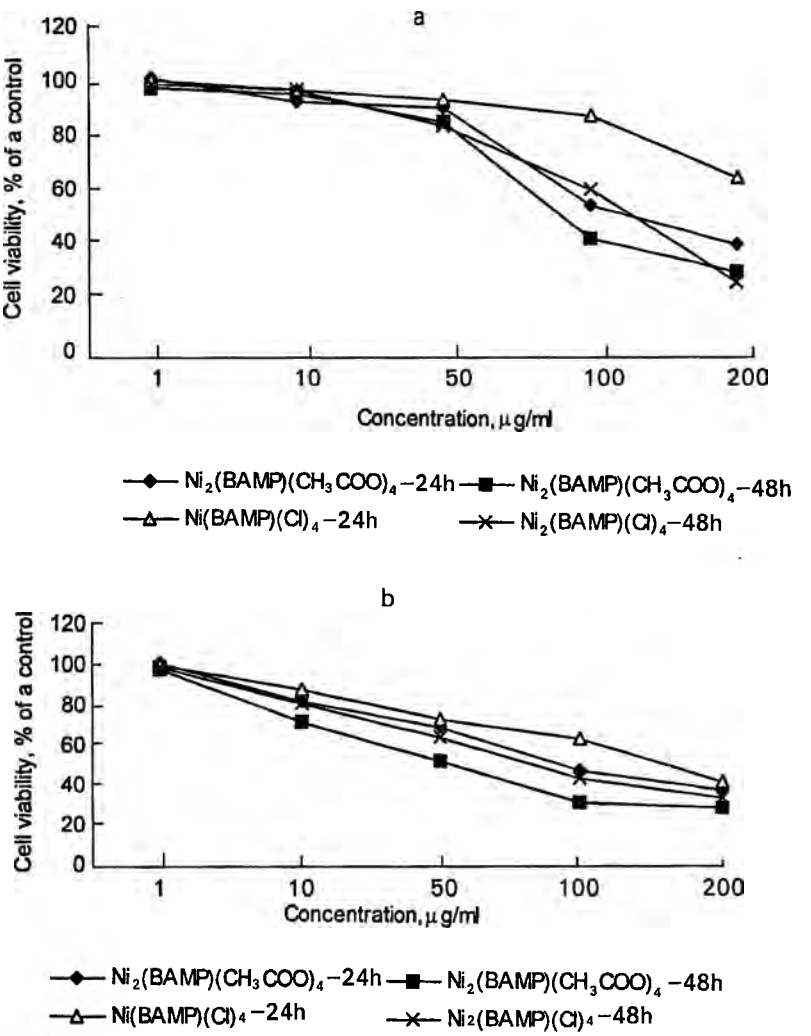

Fig. 3. Cytotoxic activity of Ni(II) complexes with BAMP on virus-transformed LSR-SF(SR) (a) and LSCC-SF(Mc29) (b) cells

The relative viability of LSR-SF(SR) treated with 100 μg/ml $Ni_2(BAMP)(CH_3COO)_4$ or $Ni_2(BAMP)(Cl)_4$, for 48 h was estimated to be 45.18% ± 1.86 and 64.09% ± 2.42 ($P < 0.01$), respectively. LSCC-SF(Mc29) tumor cells were found to be more sensitive to the cytotoxic effects of the compounds tested. 50.97% ± 2.54 and 63.01% ± 3.04 ($P < 0.05$) alive LSCC-SF(Mc29) cells were found by neutral red uptake cytotoxicity assay after 48 h culturing in the presence of 50 μg/ml $Ni_2(BAMP)(CH_3COO)_4$ or $Ni_2(BAMP)(Cl)_4$, respectively.

Cytopathological changes, such as appearance of giant, vacuolized or rounded cells as well as detachment and lysis were observed in chicken hepatoma and rat sarcoma cells treated for 48 h with $Ni_2(BAMP)(CH_3COO)_4$ or $Ni_2(BAMP)(Cl)_4$ at concentrations of 100 and 200 μg/ml.

The potential antineoplastic effects of different metals and metal compounds have been under special interest during the recent years [4, 11]. In the literature there are data published about antitumor activity in vitro and in vivo of some nickel complexes with different ligands. Thus, nickel complexes of isatin hydrazones inhibit the proliferation of human leukemic cell lines TOM1 and NB4 [18]. Ni(II) complex of 1,2-naphthoquinone-2-thiosemicarbazone was observed to be more effective in inhibiting the in vitro growth of human breast adenocarcinoma MCF-7 cells than commercial antitumor agent etoposide [6]. Nickel (II) complex of [1,4,7] triazecan-9-ol was found to induce DNA damages and apoptosis in human hepatoma BEL-7402 cells [12]. Ni(II) complex of 5-methyl-2-furaldehyde thiosemicarbazone ligand was reported to be toxic for human colon adenocarcinoma cells Caco-2 [10]. While Ni(II) complex with S-methyl-N-(I-isoquinolyl) methylendithiocarbazate expressed significant activity against P388 lymphocytic leukemia test system in mice [15], Ni(II) complexes of bis(diphenylphosphino) ethane or 3-and 5-substituted salicylaldehyde 2-pyridinylhydrazones were observed to be not active in experiments with the same experimental tumor model [14, 16].

The results presented here indicated that: 1) Ni(II) complexes with BAMP - $Ni_2(BAMP)(CH_3COO)_4$ and $Ni_2(BAMP)(Cl)_4$, express time- and dose-dependent toxicity on LSCC-SF(Mc29) and LSR-SF(SR) virus-transformed cells. $Ni_2(BAMP)(CH_3COO)_4$ appears to be more toxic than $Ni_2(BAMP)(Cl)_4$; 2) Ni(II) complexes with TAMEN – $Ni(TAMEN)(ClO_4)_2$ and $Ni(TAMEN)(NCS)_2$, have no significant toxic effect on tumor cell lines investigated administered up to concentrations of 200 μg/ml; 3) Applied at doses examined both ligands – N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) do not reduce the viability of LSCC-SF(Mc29) and LSR-SF(SR) virus-transformed cells.

Ni(II) complexes examined in our experiments differ from each other in ligand (BAMP or TAMEN) and anion (NCS^-, Cl^-, ClO^-_4, CH_3COO^-). Each of these components (ligands and anions) as well as metal ions influences in different way physicochemical and biological properties of the complexes obtained which could explain the differences in their cytotoxic effects.

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