Trakia Journal of Sciences, Vol. 8, Suppl. 2, pp 73-77, 2010

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

INFLUENCE OF NEWLY SYNTHESIZED MIXED LIGAND IRON COMPLEXES ON VIABILITY AND PROLIFERATION OF TUMOR CELLS

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ABSTRACT

PURPOSE: The aim of the study presented here was to evaluate the putative cytotoxic and antiproliferative activities of two mixed ligand iron (II, III) complexes containing Mannich base N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) and pyridine (py, C_5H_5N) as a co-ligand.

MATERIALS AND METHODS: The following permanent cell lines were used as experimental models in our investigations: LSCC-SF-Mc29 (chicken hepatoma); LSR-SF-SR (rat sarcoma) and 8 MGBA (human glioblastoma multiforme). The influence of the compounds on cell viability and proliferation was examined by neutral red uptake cytotoxicity assay and colony-forming method.

RESULTS: The results obtained revealed that the examined mixed ligand iron complexes express low cytotoxic and antiproliferative activities when applied at concentrations of 10-200 µg/ml for 24 to 48 h. Tested independently, the ligand BAMP was no or very low toxic at the concentrations examined.

CONCLUSION: Based on their sensitivity to the toxic effects of the tested compounds, the cell lines used in our experiments are graded as follows: LSCC-SF(Mc29) > LSR-SF(SR) > 8 MGBA.

Key words: Mannich bases, pyrazolone, iron, metal complexes, cytotoxic/antiproliferative activity, tumor cell lines

INTRODUCTION

In 1912 Mannich and Krösche discovered the property of formaldehyde to bind an amine with a carbon acid *via* a methylene bridge (1). This method was utilized to obtain pharmaceutical products by implication of acid 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. We found in the previous investigations that some copper (I, II) (2,3), cobalt (II) (4,5), iron (II, III) (6) and nickel (II) (7) complexes with N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) significantly decreased viability and proliferation of cultured animal and human cell lines. On the other hand, iron is known to be essential for fundamental cell functions, such as DNA synthesis, transport of oxygen and electrons, and cell respiration (8-10).

The aim of the present study was to evaluate the effect of two newly synthesized Fe(II, III) mixed ligand complexes containing the above mentioned Mannich base BAMP as a ligand as well as pyridine (py) as a coligand on viability and proliferation of cultured tumor cells.

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MATERIALS AND METHODS

Compounds:

The experiments were performed with two mixed ligand iron (II, III) complexes containing an antipyrine moiety like the Mannich-bases N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP, **Figure 1**) and pyridine (py, C_5H_5N) as a coligand: $Fe_2BAMPpy_2Cl_4$ (TS21) and $Fe_2BAMPpy_2Cl_6$ (TS22). The mixed ligand iron complexes were obtained according to the previous work (11).

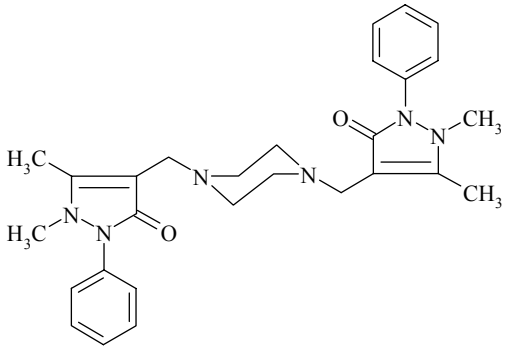

Figure 1. N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP)

The ligand BAMP was also included in the experiments for comparative investigations.

The compounds were initially dissolved in dimethylsulfoxide (DMSO, Serva) and then diluted in culture medium. The final concentration of DMSO in the stock solutions (where the concentration of the tested compound was 1 mg/mL) was 2%. The stock solutions were stored at 4°C and used in the experiments no longer than two weeks after their preparation.

Cell cultures and cultivation:

The following cell lines were used as model systems in our study: LSCC-SF(Mc29), established from a transplantable chicken hepatoma induced by the myelocytomatosis virus Mc29 (12), LSR-SF(SR), derived from a transplantable sarcoma in rat induced by Rous sarcoma virus strain Schmidt-Ruppin (SR-RSV) (13), 8 MGBA (human glioblastoma multiforme) (14).

Cells were grown as monolayer cultures in a combination (1 : 1/ vol. : vol) 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 x 10^4 cells/well. At the 24th h the cells from the monolayer were washed and covered with media modified with different concentrations of the tested compounds (each concentration in 6 to 8 repetitions). Samples of cells, grown in a non-modified medium, served as control. Effect of the compounds on cell viability was evaluated by neutral red uptake (NR) cytotoxicity assay (15) after 24 or 48 h of incubation. Optical density was measured at wave length 540 nm by Organon Teknika Reader 530. Relative cell viability, expressed as a percentage of the untreated control, was calculated for each concentration. All data points represent an average of at least three independent assays.

Colony-forming assay:

Tumor cells (approximately 10^3 cells/well) suspended in 0.45% purified agar (Difco) in medium containing different concentrations (ranging from 1 to 200 μg/ml) of metal complexes or ligands and layered in 24 well microplates (Cellstar). The presence/absence of colonies was registered using an inverted microscope (Carl Zeiss, Jena, Germany) during 16-day period.

Statistical analysis:

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

RESULTS

Neutral red uptake cytotoxicity assay:

Applied independently at concentrations of 1, 10, 50, 100 and 200 μg/ml, BAMP did not significantly reduce the viability of tumour cells investigated - more than 95% of LSR-SF(SR) and 8MGBA, and > 90% of LSCC-SF(Mc29) cells cultivated in the presence of BAMP were found to be alive after 24 h and 48 h of treatment.

Data obtained by neutral red uptake assay regarding the effects of mixed ligand iron (II, III) complexes on cell viability are summarized in **Tables 1, 2** and **3**. The CC_{50} values (concentration which reduces cell viability by 50%) were calculated only for chicken hepatoma cells treated for 48 h with TS21 (CC_{50} = 174 ± 4.6) and TS22 (CC_{50} = 183 ± 5.6). In all the other

cases the viability of the cells cultured for 24 h and 48 h in the presence of the tested compounds at concentrations up to 200 μg/ml was higher than 50 %.

Based on their sensitivity to the toxic effects of the examined compounds, the cell lines used in our experiments are graded as follows: LSCC-SF(Mc29) > LSR-SF(SR) > 8 MGBA.

Table 1. *Effect of mixed ligand iron complexes TS21 = $Fe_2BAMPpy_2Cl_4$ and TS22 = $Fe_2BAMPpy_2Cl_6$ on viability of LSCC-SF(Mc29) chicken hepatoma cells*

CONCENTRATION (μg/ml)	TS21		TS22	
	24 h	48 h	24 h	48 h
10	102 ± 3.15	97 ± 7.24	98 ± 3.13	97 ± 6.94
100	98 ± 5.26	67 ± 6.59**	94 ± 7.03	70 ± 5.48**
200	98 ± 6.63	43 ± 5.84**	89 ± 4.86	46 ± 3.15**

** $P < 0.01$

Table 2. *Effect of mixed ligand iron complexes TS21 = $Fe_2BAMPpy_2Cl_4$ and TS22 = $Fe_2BAMPpy_2Cl_6$ on viability and proliferation of LSR-SF(SR) rat sarcoma cells*

CONCENTRATION (μg/ml)	TS21		TS22	
	24 h	48 h	24 h	48 h
10	99 ± 1.84	100 ± 2.43	99 ± 3.26	97 ± 3.08
50	100 ± 2.19	97 ± 3.16	96 ± 2.54	97 ± 4.06
100	94 ± 3.15	69 ± 2.24**	97 ± 1.97	95 ± 3.14
200	88 ± 2.29*	66 ± 2.29**	95 ± 2.93	94 ± 2.63

* $P < 0.05$, ** $P < 0.01$

Table 3. *Effect of mixed ligand iron complexes TS21 = $Fe_2BAMPpy_2Cl_4$ and TS22 = $Fe_2BAMPpy_2Cl_6$ on viability of 8 MGBA human glioblastoma cells*

CONCENTRATION (μg/ml)	TS21		TS22	
	24 h	48 h	24 h	48 h
10	97 ± 4.53	97 ± 5.86	96 ± 7.93	95 ± 2.54
50	99 ± 4.13	96 ± 6.08	97. ± 5.16	97 ± 3.93
100	94 ± 3.84	93 ± 4.74	96 ± 4.23	92 ± 4.53
200	91 ± 4.93	87 ± 4.15*	93 ± 4.86	93 ± 5.06

* $P < 0.05$

Effect on colony-forming ability of tumor cells:

The compounds investigated were found to be unable to prevent the growth of tumor cells in semi-solid mediuim, when applied at concentrations of 10-200 μg/ml.

DISCUSSION

In the literature there are data that different iron containing compounds possess antintumor properties *in vitro* and *in vivo* (16-19). The results obtained by us in this study reveal that the examined mixed ligant Fe(II, III) complexes express low cytotoxic and antiproliferative activities when applied at concentrations of 10 to 200 μg/ml for 24 and 48 h. Among the cell lines used as experimental models in our investigations, the chicken hepatoma cells LSCC-SF(Mc29) were found to be the most sensitive to the cytotoxic and antiproliferative effects of the tested compounds.

In our previous studies we examined the cytotoxic and antiproliferative activity of three other groups of metal complexes: a) iron (II, III) complexes with TAMEN or BAMP with molecular formulas ($Fe_2(BAMP)Cl_6$; $Fe_2(TAMEN)Cl_6$; $Fe_2(BAMP)Cl_4$; $Fe(TAMEN)(NO_3)_3$; $Fe(BAMP)(NO_3)_3$) (6); b) mixed ligand complex of Cu(II) with molecular formula $Cu_2BAMPdipyCl_4$, (3),

where dipy = 2,2 dipyridyl; and c) the complex of cobalt(II) - $Co_2BAMPpy_2Cl_4$ (5). All above mentioned groups of metal complexes were found to be relatively more pronounced cytotoxic and antiproliferative agents (especially $Fe_2(TAMEN)Cl_6$) as compared to the mixed ligand Fe(II, III) complexes. According to their cytotoxic and antiproliferative properties, the mixed ligand complexes of copper, cobalt and iron are graded as follows: Cu > Co > Fe.

Independently tested both ligands – N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN) exerted no cytotxic nor antiproliferative activities applied at the same concentrations as those used in our experiments. On the other hand, the metal (Cu, Co, Fe) complexes express different cytotoxic and antiproliferative activities. This is not surprising because these compounds differ from each other in metal ion, ligand/s (BAMP, TAMEN, py, dipy) and anion (NO_3^-, Cl^-). Each of these components influences in different way physicochemical and biological properties of the complexes, which could explain the differences in their cytotoxic and antiproliferative effects. Further investigations are underway to clarify the structure-activity relationship and mechanism(s) of action of metal complexes with Mannich bases.

ACKNOWLEDGEMENTS

This work was supported by Grant DO-02-39/2009 from the National Science Fund, Sofia, Bulgaria.

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